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(54) Methods and products for facile microbial expression of DNA sequences.

(57) Microbial expression of an exogenous polypeptide may be inefficient if the messenger RNA has secondary structure (due to complementarity of spaced regions of the molecule) which impedes interaction with a ribosome. The occurrence of such structure can be predicted. The degeneracy of the genetic code gives freedom to alter the nucleic acid composition of the cDNA to eliminate harmful secondary structure of the mRNA.

For example, bovine growth hormone (BGH) is poorly expressed by *E. coli* transformed with a recombinant plasmid containing the natural BGH gene. The corresponding mRNA has complementary regions at 46–51 and 73–78. Therefore a plasmid pBGH 33-4 was constructed containing a BGH gene whose upstream portion was synthesized (making use of plasmid pHGH 207-1 which contains an efficiently expressible gene for the closely related human growth hormone) so as not to cause the troublesome complementarity. *E. coli* transformed therewith produced BGH with a yield of better than 10^5 copies per cell.

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METHODS AND PRODUCTS FOR FACILE MICROBIAL EXPRESSION OF DNA SEQUENCES

The present invention provides methods and means for preparing DNA sequences that provide messenger RNA having improved translation characteristics. The resulting messenger RNA may be highly efficient in translation to give substantial amounts of polypeptide product that is normally heterologous to the host microorganism. The DNA sequences which are ultimately expressed, that is, transcribed into messenger RNA (mRNA) which is in turn translated into polypeptide product, are, in essential part, synthetically prepared, in accordance with this invention, utilizing means that favor the substantial reduction or elimination of secondary and/or tertiary structure in the corresponding transcribed mRNA. An absence or substantial reduction in such secondary/tertiary structure involving the 5' end of mRNA permits effective recognition and binding of

ribosomes(s) to the mRNA for subsequent translation. Thus, the efficiency of translation is not hindered or impaired by conformational impediments in the structure of the transcribed mRNA. Methods and means for measuring mRNA secondary/tertiary structure are also described as well as associated means designed to insure that secondary/tertiary structure is kept below certain preferred limits. This invention is exemplified by the preparation of various preferred protein products.

With the advent of recombinant DNA technology, the controlled microbial production of an enormous variety of useful polypeptides has become possible, putting within reach the microbially directed manufacture of hormones, enzymes, antibodies, and vaccines useful against a wide variety of diseases. Many mammalian polypeptides, such as human growth hormone and leukocyte interferons, have already been produced by various microorganisms.

One basic element of recombinant DNA technology is the plasmid, an extrachromosomal loop of double-stranded DNA found in bacteria oftentimes in multiple copies per cell. Included in the information encoded in the plasmid DNA is that required to reproduce the plasmid in daughter cells (i.e., a "replicon") and ordinarily, one or more selection characteristics, such as resistance to antibiotics, which permit clones of the host cell containing the plasmid of interest to be recognized and preferentially grown in selective media. The utility of such bacterial plasmids lies in the fact that they can be specifically cleaved by one or

another restriction endonuclease or "restriction enzyme", each of which recognizes a different site on the plasmidic DNA. Heterologous genes or gene fragments may be inserted into the plasmid by endwise joining at the cleavage site or at reconstructed ends adjacent to the cleavage site. (As used herein, the term "heterologous" refers to a gene not ordinarily found in, or a polypeptide sequence ordinarily not produced by, a given microorganism, whereas the term "homologous" refers to a gene or polypeptide which is found in, or produced by the corresponding wild-type microorganism.) Thus formed are so-called replicable expression vehicles.

DNA recombination is performed outside the microorganism, and the resulting "recombinant" plasmid can be introduced into microorganisms by a process known as transformation and large quantities of the heterologous gene-containing recombinant plasmid are obtained by growing the transformant. Moreover, where the gene is properly inserted with reference to portions of the plasmid which govern the transcription and translation of the encoding DNA, the resulting plasmid can be used to actually produce the polypeptide sequence for which the inserted gene codes, a process referred to as expression. Plasmids which express a (heterologous) gene are referred to as replicable expression vehicles.

Expression is initiated in a DNA region known as the promotor. In some cases, as in the lac and trp systems discussed infra, promotor regions are overlapped by "operator" regions to form a combined promotor-operator. Operators are DNA sequences which are recognized by so-called repressor proteins which serve to regulate the frequency of transcription initiation from a particular promoter. In the

trancription phase of expression, RNA polymerase recognizes certain sequences in and binds to the promoter DNA. The binding interaction causes an unwinding of the DNA in this region, exposing the DNA as a template for synthesis of messenger RNA. The messenger RNA serves as a template for ribosomes which bind to the messenger RNA and translate the mRNA into a polypeptide chain having the amino acid sequence for which the RNA/DNA codes. Each amino acid is encoded by a nucleotide triplet or "codon" which collectively make up the "structural gene", i.e., that part of the DNA sequence which encodes the amino acid sequence of the expressed polypeptide product.

After binding to the promoter, RNA polymerase initiates the transcription of DNA encoding a ribosome binding site including a translation initiation or "start" signal (ordinarily ATG, which in the resulting messenger RNA becomes AUG), followed by DNA sequences encoding the structural gene itself. So-called translational stop codons are transcribed at the end of the structural gene whereafter the polymerase may form an additional sequence of messenger RNA which, because of the presence of the translational stop signal, will remain untranslated by the ribosomes.

Ribosomes bind to the binding site provided on the messenger RNA, in bacteria ordinarily as the mRNA is being formed, and direct subsequently the production of the encoded polypeptide, beginning at the translation start signal and ending at the previously mentioned stop signal(s). The resulting product may be obtained by lysing the host cell and recovering the product by appropriate purification from other bacterial proteins.

Polypeptides expressed through the use of recombinant DNA technology may be entirely heterologous, functional proteins, as in the case of the direct expression of human growth hormone, or alternatively may comprise a bioactive heterologous polypeptide portion and, fused thereto, a portion of the amino acid sequence of a homologous polypeptide, as in the case of the production of intermediates for somatostatin and the components of human insulin. In the latter cases, for example, the fused homologous polypeptide comprised a portion of the amino acid sequence for beta galactosidase. In those cases, the intended bioactive product is rendered bioinactive within the fused, homologous/ heterologous polypeptide until it is cleaved in an extracellular environment. Fusion proteins like those just mentioned can be designed so as to permit highly specific cleavage of the precusor protein from the intended product, as by the action of cyanogen bromide on methionine, or alternatively by enzymatic cleavage. See, eg., G.B. Patent Publication No. 2 007 676 A.

If recombinant DNA technology is to fully sustain its promise, systems must be devised which optimize expression of gene inserts, so that the intended polypeptide products can be made available in controlled environments and in high yields.

Promoter Systems

As examples, the beta lactamase and lactose promoter systems have been advantageously used to initiate and sustain microbial production of heterologous polypeptides. Details relating to the make-up and construction of these promoter systems have been published by Chang et al., Nature 275, 617 (1978) and Itakura et al., Science 198, 1056 (1977), which are

hereby incorporated by reference. More recently, a system based upon tryptophan, the so-called trp promoter system, has been developed. Details relating to the make-up and construction of this system have been published by Goeddel et al., Nucleic Acids Research 8, 4057 (1980) and Kleid et al., U.S.S.N. 133, 296, filed March 24, 1980, (or the equivalent European Patent Publication 0036776) which are hereby incorporated by reference. Numerous other microbial promoters have been discovered and utilized and details concerning their nucleotide sequences, enabling a skilled worker to ligate them functionally within plasmid vectors, have been published -- see, e.g. Siebenlist et al., Cell 20, 269 (1980), which is incorporated herein by this reference.

Historically, recombinant cloning vehicles (extrachromosomal duplex DNA having, inter alia., a functional origin of replication) have been prepared and used to transform microorganisms -- cf. Ullrich et al., Science 196, 1313 (1977). Later, there were attempts to actually express DNA gene inserts encoding a heterologous polypeptide. Itakura et al. (Science 198, 1056 (1977)) expressed the gene encoding somatostatin in E. coli. Other like successes followed, the gene inserts being constructed by organic synthesis using newly refined technology. In order, among other things, to avoid possible proteolytic degradation of the polypeptide product within the microbe, the genes were ligated to DNA sequences coding for a precursor polypeptide. Extracellular cleavage yielded the intended protein product, as discussed above.

In the case of larger proteins, chemical synthesis of the underlying DNA sequence proved unwieldy. Accordingly, resort was had to the preparation of gene sequences by reverse transcription from corresponding messenger RNA obtained from requisite tissues and/or culture cells. These methods did not always prove satisfactory owing to the termination of transcription short of the entire sequence; and/or the desired sequence would be accompanied by naturally occurring precursor leader or signal DNA. Thus, these attempts often have resulted in incomplete protein product and/or protein product in non-cleavable conjugate form -- cf. Villa-Komaroff et al., Proc. Natl. Acad. Sci. (USA) 75, 3727 (1978) and Seeburg et al., Nature 276, 795 (1978).

In order to avoid these difficulties, Goeddel et al., Nature 281, 544 (1979), constructed DNA, inter alia encoding human growth hormone, using chemically synthesized DNA in conjunction with enzymatically synthesized DNA. This discovery thus made available the means enabling the microbial expression of hybrid DNA (combination of chemically synthesized DNA with enzymatically synthesized DNA), notably coding for proteins of limited availability which would probably otherwise not have been produced economically. The hybrid DNA (encoding heterologous polypeptide) is provided in substantial portion, preferably a majority, via reverse transcription of mRNA, while the remainder is provided via chemical synthesis. In a preferred embodiment, synthetic DNA encoding the first 24 amino acids of human growth hormone (HGH) was constructed according to a plan which incorporated an endonuclease restriction site in the DNA corresponding to HGH amino acids 23 and 24. This was done to facilitate a connection with downstream HGH cDNA sequences. The various 12 oligonucleotide long fragments making up the synthetic part of

the DNA were chosen following then known criteria for gene synthesis: avoidance of undue complementarity of the fragments, one with another, except, of course, those destined to occupy opposing sections of the double stranded sequence; avoidance of AT rich regions to minimize transcription termination; and choice of "microbially preferred codons." Following synthesis, the fragments were permitted to effect complementary hydrogen bonding and were ligated according to methods known per se. This work is decribed in published British Patent Specification 2055382 A, which corresponds to Goeddel et al., U.S.S.N. 55126, filed July 5, 1979 which is hereby incorporated by this reference.

While the successful preparation and expression of such hybrid DNA provided a useful means for preparing heterologous polypeptides, it did not address the general problem that eucaryotic genes are not always recognized by procaryotic expression machinery in a way which provides copious amounts of end product. Evolution has incorporated sophistication unique to discrete organisms. Bearing in mind that the eucaryotic gene insert is heterologous to the procarytic organism, the relative inefficiency in expression often observed can be true for any gene insert whether it is produced chemically, from cDNA or as a hybrid. Thus, the criteria used to construct the synthetic part of the gene for HGH, defined above, are not the sole factors influencing expression levels. For example, concentrating on codon choice as the previous workers have done--cf. British Patent Specification 2007676 A -- has not been completely successful in raising the efficiency of expression towards maximal expression levels.

Guarante et al., Science 209, 1428 (1980) experimented with several hybrid ribosome binding sites, designed to match the number of base pairs between the Shine-Dalgarno sequence and the ATG of some known E. coli binding sites, their work suggesting that the reason(s) for observed relatively low efficiencies of eucaryotic gene expression by procaryote organisms is more subtle.

That the initiation of mRNA translation may be a multicomponent process is illustrated by work reported by Iserentant and Fiers, Gene 9, 1 (1980). They postulate that secondary structure of mRNA is one of the components influencing translation efficiency and imply that the initiation codon and ribosome interaction site of secondary structured, folded mRNA must be "accessible." However, what those workers apparently mean by "accessible" is that the codon and site referred to be located on the loop, rather than the stem, of the secondary structure models they have hypothesized.

The present invention is based upon the discovery that the presence of secondary/tertiary conformational structure in the mRNA interferes with the initiation and maintenance of ribosomal binding during the translation phase of heterologous gene expression.

The present invention, relating to these findings, uniquely provides methods and means for providing efficient expression of heterologous gene inserts by the requisite microbial host. The present invention is further directed to a method of microbially producing heterologous polypeptides, utilizing specifically tailored heterologous gene inserts in microbial expression

vehicles, as well as associated means. It is particularly directed to the use of synthetically derived gene insert portions that are prepared so as to both encode the desired polypeptide product and provide mRNA that has minimal secondary/ tertiary structure and hence is accessible for efficient ribosomal translation.

In preferred embodiments of the present invention, synthetic DNA is provided for a substantial portion of the initial coding sequence of a heterologous gene insert, and optionally, upstream therefrom through the ATG translational start codon and ribosome binding site. The critical portion of DNA is chemically synthesized, keeping in mind two factors: 1) the creation of a sequence that codes for the initial (N-terminal) amino acid sequence of a polypeptide comprising a functional protein or bioactive portion thereof and 2) the assurance that said sequence provides, on transcription, messenger RNA that has a secondary/tertiary conformational structure which is insufficient to interfere with its accessibility for efficient ribosomal translation, as herein defined. Such chemical synthesis may use standard organic synthesis using modified mononucleotides as building blocks such as according to the method of Crea et al., Nucleic Acids Research 8, 2331 (1980) and/or the use of site directed mutagenesis of DNA fragments such as according to the method of Razin et al., Proc. Natl. Acad Sci (USA) 75, 4268 (1978) and/or synthetic primers on certain appropriately sequenced DNA fragments followed by specific cleavage of the desired region.

The present invention is directed to a process of preparing DNA sequences comprising nucleotides arranged sequentially so as to encode the proper amino acid sequence of a given polypeptide.

This method may involve obtaining a substantial portion of the DNA coding sequence of a given polypeptide via means other than chemical synthesis, most often by reverse transcription from requisite tissue and/or culture cell messenger RNA. This fragment encodes the C-terminal portion of the polypeptide and is ligated, in accordance herewith, to a remainder of the coding sequence, e.g. obtained by chemical synthesis, optionally including properly positioned translational start and stop signals and upstream DNA through the ribosome binding site and the first nucleotide (+1) of the resultant messenger RNA. The synthetic fragment is designed by nucleotide choice dependent on conformation of the corresponding messenger RNA according to the criteria as herein discussed.

The thus prepared DNA sequences are suited for insertion and use in replicable expression vehicles designed to direct the production of the heterologous polypeptide in a transformant microorganism. In these vehicles, the DNA sequence is operably linked to promotor systems which control its expression. The invention is further directed to the replicable expression vehicles and the transformant microorganisms so produced as well as to cultures of these microorganisms in fermentation media. This invention is further directed to associated methods and means and to specific embodiments for the directed production of messenger RNA transcripts that are accessible for efficient ribosomal translation.

Specifically excluded from the present invention is the hybrid DNA encoding human growth hormone (HGH) as disclosed by Goeddel et al., Nature 281, 544 (1979). While this particular hybrid DNA was successfully expressed to produce the intended product, the concept of the present invention was not appreciated by these workers (and hence not taught by them) and consequently was not

practised in the fortuitous preparation of their expressible hybrid DNA for HGH. This hybrid DNA has the following sequence (Table 1):

Table I

```
     1
met phe pro thr ile pro leu ser arg leu phe asp asn ala met
ATG TTC CCA ACT ATA CCA CTA TCT CGT CTA TTC GAT AAC GCT ATG

                        20
leu arg ala his arg leu his gln leu ala phe asp thr tyr gln
CTT CGT GCT CAT CGT CTT CAT CAG CTG GCC TTT GAC ACC TAC CAG

                                                40
glu phe glu glu ala tyr ile pro lys glu gln lys tyr ser phe
GAG TTT GAA GAA GCC TAT ATC CCA AAG GAA CAG AAG TAT TCA TTC

leu gln asn pro gln thr ser leu cys phe ser glu ser ile pro
CTG CAG AAC CCC CAG ACC TCC CTC TGT TTC TCA GAG TCT ATT CCG

 60
thr pro ser asn arg glu glu thr gln gln lys ser asn leu glu
ACA CCC TCC AAC AGG GAG GAA ACA CAA CAG AAA TCC AAC CTA GAG

                        80
leu leu arg ile ser leu leu leu ile gln ser trp leu glu pro
CTG CTC CGC ATC TCC CTG CTG CTC ATC CAG TCG TGG CTG GAG CCC

                                                100
val gln phe leu arg ser val phe ala asn ser leu val tyr gly
GTG CAG TTC CTC AGG AGT GTC TTC GCC AAC AGC CTA GTG TAC GGC

ala ser asp ser asn val tyr asp leu leu lys asp leu glu glu
GCC TCT GAC AGC AAC GTC TAT GAC CTC CTA AAG GAC CTA GAG GAA

120
gly ile gln thr leu met gly arg leu glu asp gly ser pro arg
GGC ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCC CGG

                        140
thr gly gln ile phe lys gln thr tyr ser lys phe asp thr asn
ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC AAG TTC GAC ACA AAC

                                                160
ser his asn asp asp ala leu leu lys asn tyr gly leu leu tyr
TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC GGG CTG CTC TAC

cys phe arg lys asp met asp lys val glu thr phe leu arg ile
TGC TTC AGG AAG GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC

180                                                     191
val gln cys arg ser val glu gly ser cys gly phe stop
GTG CAG TGC CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG
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The chemically synthetic DNA sequences hereof extend preferably from the ATG translation initiation site, and optionally upstream therefrom a given distance, to or beyond the transcription initiation sice (labelled +1 by convention), and to sequences downstream encoding a substantial part of the desired polypeptide. By way of preference, the synthetic DNA comprises upwards of approximately 75 or more nucleotide pairs of the structural gene representing about the proximal (N-terminal) 25 amino acids of the intended polypeptide. In particularly preferred embodiments, the synthetic DNA sequence extends from about the translation initiation site (ATG) to about nucleotide 75 of the heterologous gene. In alternative terms, the synthetic DNA sequence comprises nucleotide pairs from +1 (transcription initiation) to about nucleotide 100 of the transcript.

Because of the degeneracy of the genetic code, there is substantial freedom in codon choice for any given amino acid sequence. Given this freedom, the number of different DNA nucleotide sequences encoding any given amino acid sequence is exceedingly large, for example, upwards of 2.6×10^5 possibilities for somatostatin consisting of only 14 amino acids. Again, the present invention provides methods and means for selecting certain of these DNA sequences, those which will efficiently prepare functional product. For a given polypeptide product hereof, the present invention provides means to select, from among the large number possible, those DNA sequences that provide transcripts, the conformational structure of which admits of accessiblity for operable and efficient ribosomal translation.

Conformational structure of mRNA transcripts is a consequence of hydrogen bonding between complementary nucleotide sequences that may be separated one from another by a sequence of noncomplementary

nucleotides. Such bonding is commonly referred to as secondary structure. So-called tertiary structures may add to the conformation of the overall molecule. These structures are believed to be a result of spatial interactions within one or more portions of the molecule -- so-called stacking interactions. In any event, the conformational structure of a given mRNA molecule can be determined and measured. Furthermore, we have now discovered that certain levels of conformational structure of mRNA transcripts interfere with efficient protein synthesis, thus effectively blocking the initiation and/or continuation of translation (elongation) into polypeptide product. Accordingly, levels at which such conformational structure does not occur, or at least is minimal, can be predicted. Nucleotide choice can be prescribed on the basis of the predictable, permissible levels of conformational structure, and preferred gene sequences determined accordingly.

The measurement of mRNA conformational structure is determined, in accordance herewith, by measuring the energy levels associated with the conformational structure of the mRNA molecule.

In determining such energy levels, the thermodynamic disassociation energy connected with one or a series of homologous base pairings is calculated, for example according to the rules of Tinoco et al., Nature New Biol 246, 40(1973). In this calculation, AT base pairing is assigned an associated energy level of about -1.2 Kcal/mole while a CG base pairing is assigned an associated energy level of about -2 Kcal/mole. Adjacent homologous pairings are more than additive, doubtless due to stacking interactions and other associative factors. In any event, it has been determined that in those instances where regional base pairing interactions result in energy levels stronger than about -12 kcal/mole (that is,

values expressed arithmetically in numbers less than about -12 kcal/mole) for a given homologous sequence, such interactions are likely sufficient to hinder or block the translation phase of expression, most probably by interfering with accessibility for necessary ribosomal binding.

A given DNA sequence is screened as follows: A first series of base pairs, e.g., approximately the first six base pairs, are compared for homology with the corresponding reverse last base pairs of the gene. If such homology is found, the associate energy levels are calculated according to the above considerations. The first series of base pairs is next compared with the corresponding last base pairs up to the penultimate base pair of the gene and the associative energy levels of any homology calculated. In succession the first series of base pairs is next compared with the corresponding number of base pairs up to the antepenultimate base pair, and so on until the entire gene sequence is compared, back to front. Next, the series of base pairs beginning one downstream from the first series, e.g. base pairs 2 to 7 of the prior example, is compared with the corresponding number from the end and progressively toward the front of the gene, as described above. This procedure is repeated until each base pair is compared for homology with all other regions of the gene and associated energy levels are determined. Thus, for example in Figure 3 there are provided results of such scanning and calculating for two genes - those encoding natural bovine growth hormone (BGH) and synthetic (i.e., hybrid) BGH. It can be seen that natural BGH contains two regions of homology considered relevant herein (i.e., energy level greater than about -12 kcal/mole), to wit, six base pairs from base pair 33 to 38 with homologous pairs 96 to 101 and six base pairs from 46 to 51 with 73 to 78. The first is not significant for present purpose,

despite the energy level (-15.40 kcal/mole), presumably because the region of homology lies downstream a sufficient distance so as not to be influential on translation efficiency. The second region is significant as evidenced by the poor yields of product as described herein cf. infra. The synthetic BGH gene where such region of homology was eliminated provided good yields of intended protein.

An embodiment of the present invention will now be described by way of example with reference to the accompanying drawings, in which:

Figure 1 depicts the amino acid and nucleotide sequences of the proximal portions of natural BGH, synthetic HGH, and synthetic BGH. The amino acids and nucleotides in natural BGH that are different from those in synthetic HGH are underlined. The nucleotides in the proximal portion of the synthetic BGH gene that differ from those in the natural BGH gene also are underlined. The position of the PVUII restriction site at the end of the proximal portion of these genes is indicated.

In arriving at the synthetic BGH gene encoding the proper amino acid sequence for BGH, the nucleotide sequences of natural BGH and synthetic HGH were compared. Nucleotide selections were made based upon the synthetic HGH gene for construction of the synthetic BGH gene taking into account also the latitude permitted by the degeneracy of the genetic code, using a minimum of nucleotide changes from the synthetic HGH sequence.

Figure 2 depicts the nucleotide sequences of the sense strands of both natural and synthetic BGH genes along with the transcribed portions of the respective preceding trp-promotor sequences. The first nucleotide of each transcript is indicated as "+1" and the following nucleotides are numbered sequentially. The sequences

are lined up to match the translated coding regions of both genes, beginning at the start codon "ATG" of each (overlined). The transcript of the natural BGH gene shows an area of "secondary structure" due to interactions of nucleotides 46 to 51 with nucleotides 73 to 78, respectively (see Figure 3), thus creating the stem-loop structure depicted. This area is not present in the synthetic BGH gene, removed by virtue of nucleotide changes (see Figure 1), which nevertheless retains the correct amino acid sequence.

Figure 3 shows the locations and stabilities of secondary structures in the transcripts of natural and synthetic BGH. (See Figure 2) These locations and stabilities were determined using a nucleotide by nucleotide analysis, as described herein. Each area of significant secondary structure of each proximal portion of gene is listed in the respective table. Thus, for natural BGH versus synthetic BGH, it is noted that the energy levels of "secondary structure" at corresponding portions of the translatable transcripts (namely, nucleotides 46 to 78 comprising a 6 nucleotide long stem in natural BGH versus nucleotides 52 to 84 of synthetic BGH) are markedly different (-15.2 kcal/mole versus greater than -10 kcal/mole), accounting for the observed success of expression of the synthetic BGH gene versus the natural BGH gene, cf. infra. The energy levels indicate the significance of the relative amounts of tolerable "secondary structure", i.e., values arithmetically greater than about -12kcal/mole based upon thermodynamic energy considerations. The significance of location of "secondary structure" can be appreciated by the fact that energy levels calculated for positions 33 to 101 versus 38 to 104 of natural versus synthetic BGH, respectively, did not significantly influence expression levels.

Figure 4 depicts the construction of pBGH 33 used as shown in Figure 5.

Figure 5 depicts the construction of plasmids harboring DNA sequences for hybrid polypeptides: pBHGH 33-1 used as shown in Figure 7, pBHGH being a hybrid of bovine and human growtn hormone sequences, and pHBGH a hybrid of human and bovine sequences.

Figure 6 depicts the technique used to assemble the synthetic proximal portion of the BGH gene, pBR 322-01, used in the construction shown in Figure 7.

Figure 7 depicts the construction of the plasmid (pBGH 33-3) harboring the gene for BGH comprising the synthetic proximal portion as shown in Figure 6.

Figure 8 depicts the construction of expression plasmid pBGH 33-4 harboring the hybrid BGH gene.

Figure 9 is the result of a polyacrylamide gel segregation of cell protein. Part A shows no BGH production at any cell density using the culture containing natural BGH gene. Part B shows the expression of synthetic BGH gene (lanes BGH #1 and #2) in the same medium as used for Part A. The levels of expression indicated in Part B, as opposed to Part A, reflect the production of BGH in amounts exceeding about 100 thousand copies per cell.

In its most preferred embodiment, the invention is illustrated by the microbial production of bovine growth hormone (BGH). BGH is endogenous in bovine, e.g., cattle, and is responsible for proper

physical maturation of the animal. It is also useful for increasing weight gain, feed conversion efficiency, lean to fat ratio, and milk production. Its sequence of 190 amino acids is known. See Dayhoff, Atlas of Protein Sequence and Structure 1972, National Biomedical Research Foundation, Washington, D.C. The present invention makes possible the preparation of commercial quantities of the compound, enabling now its application on a large scale in the animal husbandry industry. An initial approach toward preparing BGH microbially took advantage of a source of bovine pituitary glands. By extraction and purification, the requisite mRNA for BGH was isolated and from it, corresponding cDNA prepared. Thus, this initial work resulted in a gene corresponding, for all intents and purposes, to the natural DNA sequence of BGH. After removal of DNA coding for the presequence and adding a start codon, the cDNA was ligated to a plasmid vector under proper control of a promotor. This plasmid was used to transform E. coli host which was grown under usual conditions. The efficiency of expression of BGH product was poor, a consequence, it was discovered, of conformational structure of the messenger RNA, which greatly reduced its accessibility for ribosomal translation, cf. Figure 3.

For example, it was found that in "natural" BGH mRNA there are regions of complementary homology. One significant region centers around positions +46 to +51 with a homologous region at positions +73 to +78. Secondary structure considerations, in these two defined regions, are thought to create a hairpin arrangement just downstream from the translation start codon ATG and the ribosome binding site. This conformational arrangement interferes with or prematurely disrupts ribosomal binding, and hence, inhibits translation.

The recognition of this phenomenon prompted investigations into the nature of the DNA sequence in these regions and the discovery of methods and means to obviate the problem. In accordance herewith, advantage was taken of a Pvu II endonuclease restriction site at the BGH DNA corresponding to amino acid 24. DNA for the first 24 amino acids of BGH was chemically synthesized, the selection of nucleotides taking into strict account proper coding sequence and resultant mRNA secondary/ tertiary structure considerations. Employing the method defined above, it was found that certain nucleotide base selections would be suitable, on the basis of predicted conformational structure energy levels, to prepare gene sequences properly encoding BGH but devoid of problematic conformational structure. One of these was selected and synthesized. Ligations at the Pvu II terminus of the synthetic piece to the cDNA downstream therefrom produced the desired hybrid gene. Construction of a replicable expression vector containing said heterologous gene as an operable insert successfully resulted in efficient expression of BGH in transformed E. coli host.

The complete nucleotide (and deduced amino acid) sequence of the thus constructed hybrid BGH gene is as follows:

```
     1
met phe pro ala met ser leu ser gly leu phe ala asn ala val
ATG TTC CCA GCT ATG TCT CTA TCT GGT CTA TTC GCT AAC GCT GTT

                    20
leu arg ala gln his leu his gln leu ala ala asp thr phe lys
CTT CGT GCT CAG CAT CTT CAT CAG CTG GCT GCT GAC ACC TTC AAA

                                                40
glu phe glu arg thr tyr ile pro glu gly gln arg tyr ser ile
GAG TTT GAG CGC ACC TAC ATC CCG GAG GGA CAG AGA TAC TCC ATC

gln asn thr gln val ala phe cys phe ser glu thr ile pro ala
CAG AAC ACC CAG GTT GCC TTC TGC TTC TCT GAA ACC ATC CCG GCC

 60
pro thr gly lys asp glu ala gln gln lys ser asp leu glu leu
CCC ACG GGC AAG GAT GAG GCC CAG CAG AAA TCA GAC TTG GAG CTG

                    80
leu arg ile ser leu leu leu ile gln ser trp leu gly pro leu
CTT CGC ATC TCA CTG CTC CTC ATC CAG TCG TGG CTT GGG CCC CTG

                                                100
gln phe leu ser arg val phe thr asn ser leu val phe gly thr
CAG TTC CTC AGC AGA GTC TTC ACC AAC AGC TTG GTG TTT GGC ACC

ser asp arg val tyr glu lys leu lys asp leu glu glu gly ile
TCG GAC CGT GTC TAT GAG AAG CTG AAG GAC CTG GAG GAA GGC ATC

120
leu ala leu met arg glu leu glu asp gly thr pro arg ala gly
CTG GCC CTG ATG CGG GAG CTG GAA GAT GGC ACC CCC CGG GCT GGG

                    140
gln ile leu lys gln thr tyr asp lys phe asp thr asn met arg
CAG ATC CTC AAG CAG ACC TAT GAC AAA TTT GAC ACA AAC ATG CGC

                                                160
ser asp asp ala leu leu lys asn tyr gly leu leu ser cys phe
AGT GAC GAC GCG CTG CTC AAG AAC TAC GGT CTG CTC TCC TGC TTC

arg lys asp leu his lys thr glu thr tyr leu arg val met lys
CGG AAG GAC CTG CAT AAG ACG GAG ACG TAC CTG AGG GTC ATG AAG

180                                             190
cys arg arg phe gly glu ala ser cys ala phe stop
TGC CGC CGC TTC GGG GAG GCC AGC TGC GCA TTC TAG
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Detailed Description

Synthesis of Proximal Portion of BGH Gene

Twelve fragments, U 1-6 (upper strand) and L 1-6 (lower strand), were synthesized. Also synthesized, in order to repair the 3' end of the gene, were 2 fragments, BGH Repair (1) (upper strand) and BGH Repair (2) (lower strand).

The 14 fragments were synthesized according to the method of Crea et al., Nucleic Acids Research, 8, 2331 (1980). The syntheses of the fragments were accomplished from the appropriate solid support (cellulose) by sequential addition of the appropriate fully protected dimer - or trimer- blocks. The cycles were carried out under the same conditions as described in the synthesis of oligothymidilic acid (see Crea et al., Supra.) The final polymer was treated with base (aq. conc NH_3) and acid (80% aq. HOAC), the polymer pelleted off and the supernatant evaporated to dryness. The residue, as dissolved in 4% aq. NH_3, was washed with ether (3x) and used for the isolation of the fully deprotected fragment. Purification was accomplished by hplc on Rsil NH_2 u-particulate column. Gel electrophoretic analysis showed that each of the fragments, U,L 1-6 and BGH Repair (1) and (2), had the correct size:

Fragment	Sequence	Size
U 1	5' AAT.TCT.ATG.TTC.C 3'	13-mer
U 2	5' CAG.CTA.TGT.CTC.T 3'	13-mer
U 3	5' ATC.TGG.TCT.ATT.C 3'	13-mer
U 4	5' GCT.AAC.GCT.GTT.C 3'	13-mer
U 5	5' TTC.GTG.CTC.AGC.A 3'	13-mer
U 6	5' TCT.TCA.TCA.GCT.GA 3'	14-mer
L 1	5' ATA.GCT.GGG.AAC.ATA.G 3'	16-mer
L 2	5' ACC.AGA.TAG.AGA.C 3'	13-mer
L 3	5' CGT.TAG.CGA.ATA.G 3'	13-mer
L 4	5' GCA.CGA.AGA.ACA.G 3'	13-mer
L 5	5' ATG.AAG.ATG.CTG.A 3'	13-mer
L 6	5' AGC.TTC.AGC.TG 3'	11-mer
BGH Repair (1)	5' AA.TTC.AGC.TGC.GCA.TTC.TAG.A 3'	21-mer
BGH Repair (2)	5' AG.CTT.CTA.GAA.TGC.GCA.GCT.G 3'	21-mer

Construction of pBGH 33 (Fig. 4)

Fresh frozen bovine pituitaries were macerated and RNA was extracted by the guanidium thiocyanate method. (Harding et al., J. Biol Chem. 252 (20), 7391 (1977) and Ullrich et al., Science 196, 1313 (1977)). The total RNA extract was then passed over an oligo-dT cellulose column to purify poly A containing messenger RNA (mRNA). Using reverse transcriptase and oligo-dT as a primer, single stranded cDNA was made from the mRNA. Second strand synthesis was achieved by use of the Klenow fragment of DNA polymerase I. Following S1 enzyme treatment and acrylamide gel electrophoresis a size cut of the total cDNA (ca. 500-1500 bp) was eluted and cloned into the Pst I site of the amp^R gene of pBR 322 using traditional tailing and annealing conditions.

The pBR 322 plasmids containing cDNA were transformed into E. coli K-12 strain 294 (ATCC No. 31446). Colonies containing recombinant plasmids were selected by their resistance to tetracycline and sensitivity to ampicillin. Approximately 2000 of these clones were screened for BGH by colony hybridization.

The cDNA clones of HGH contain an internal 550 bp HaeIII fragment. The amino acid sequence of this region is very similar to the BGH amino acid sequence. This HGH HaeIII fragment was radioactively labeled and used as a probe to find the corresponding BGH sequence amongst the 2000 clones.

Eight positive clones were identified. One of these, pBGH112, was verified by sequence analysis as BGH. This full-length clone is 940 bp long containing the coding region of the 26 amino acid presequence as well as the 191 amino acid protein sequence.

In order to achieve direct BGH expression, a synthetic "expression primer" was made having the sequence 5'-ATGTTCCCAGCCATG-3'. The nucleotides in the fourth through fifteenth position are identical to the codons of the first 4 amino acids of the mature BGH protein, as determined by sequence data of pBGH 112 . Only the 5' ATG (methionine) is alien to this region of the protein. This was necessary in order to eliminate the presequence region of our BGH clone and to provide the proper initiation codon for protein synthesis. By a series of enzymatic reactions this synthetic primer was elongated on the BGH 112 cDNA insert. The primed product was cleaved with Pst I to give a DNA fragment of 270 bp containing coding information up to amino acid 90. (Figure 4) This "expression" BGH cDNA fragment was ligated into a pBR 322 vector which contained the trp promotor. This vector was derived from pLeIF A trp25 (Goeddel et al., Nature 287, 411 (1980)). The interferon cDNA was removed and the trp25-322 vector purified by gel electrophoresis. The recombinant plasmid (pBGH710) now contained the coding information for amino acids 1-90 of the mature BGH protein, linked directly to the trp promotor. This linkage was verified by DNA sequence analysis. The second half of the coding region and the 3' untranslated region was isolated from pBGH112 by PstI restriction digest and acrylamide gel electrophoresis. This "back-end" fragment of 540 bp was then ligated into pBGH710 at the site of amino acid 90. Recombinant plasmids were checked by restriction analysis and DNA sequencing. The recombinant plasmid, pBGH33, has the trp promotor directly linked via ATG with the complete DNA coding sequence for mature BGH.

Construction of pHGH 207-1

Plasmid pGM1 carries the E. coli tryptophan operon containing the deletion LE1413 (G.F. Miozzari, et al., (1978) J. Bacteriology 1457-1466)) and hence expresses a fusion protein comprising the first 6 amino acids of the trp leader and approximately the last third of the trp E polypeptide (hereinafter referred to in conjunction as LE'), as well as the trp D polypeptide in its entirety, all under the control of the trp promoter-operator system. The plasmid, 20 µg, was digested with the restriction enzyme PvuII which cleaves the plasmid at five sites. The gene fragments were next combined with EcoRI linkers (consisting of a self complementary oligonucleotide of the sequence: pCATGAATTCATG) providing an EcoRI cleavage site for a later cloning into a plasmid containing an EcoRI site. The 20 µg of DNA fragments obtained from pGM1 were treated with 10 units T_4 DNA ligase in the presence of 200 pico moles of the 5'-phosphorylated synthetic oligonucleotide pCATGAATTCATG and in 20µl T_4 DNA ligase buffer (20mM tris, pH 7.6, 0.5 mM ATP, 10 mM $MgCl_2$, 5 mM dithiothreitol) at 4°C overnight. The solution was then heated 10 minutes at 70°C to inactivate ligase. The linkers were cleaved by EcoRI digestion and the fragments, now with EcoRI ends, were separated using polyacrylamide gel electrophoresis (hereinafter "PAGE") and the three largest fragments isolated from the gel by first staining with ethidium bromide, locating the fragments with ultraviolet light, and cutting from the gel the portions of interest. Each gel fragment, with 300 microliters 0.1xTBE, was placed in a dialysis bag and subjected to electrophoresis at 100 V for one hour in 0.1xTBE buffer (TBE buffer contains: 10.8 gm tris base, 5.5 gm boric acid, 0.09 gm Na_2EDTA in 1 liter H_2O). The aqueous solution was collected from the dialysis bag,

phenol extracted, chloroform extracted and made 0.2 M sodium chloride, and the DNA recovered in water after ethanol precipitation. (All DNA fragment isolations hereinafter described are performed using PAGE followed by the electroelution method just discussed.) The trp promoter-operator-containing gene with EcoRI sticky ends was identified in the procedure next described, which entails the insertion of fragments into a tetracycline sensitive plasmid which, upon promoter-operator insertion, becomes tetracycline resistant.

Plasmid pBRH1, (R.I. Rodriguez, et al., Nucleic Acids Research 6, 3267-3287 [1979]) expresses ampicillin resistance and contains the gene for tetracycline resistance but, there being no associated promoter, does not express that resistance. The plasmid is accordingly tetracycline sensitive. By introducing a promoter-operator system in the EcoRI site, the plasmid can be made tetracycline resistant.

pBRH1 was digested with EcoRI and the enzyme removed by phenol/$CHCl_3$ extraction followed by chloroform extraction and recovered in water after ethanol precipitation. The resulting DNA molecule was, in separate reaction mixtures, combined with each of the three DNA fragments obtained as decribed above and ligated with T_4 DNA ligase as previously described. The DNA present in the reaction mixture was used to transform competent E. coli K-12 strain 294 (K. Backman et al., Proc Nat'l Acad Sci USA 73, 4174-4198 (1976) (ATCC no. 31446) by standard techniques (V. Hershfield et al., Proc Nat'l Acad Sci USA 71, 3455-3459 (1974) and the bacteria plated on LB plates containing 20 µg/ml ampicillin and 5 µg/ml tetracycline. Several tetracycline-resistant colonies were selected, plasmid DNA isolated and the presence of the desired fragment confirmed

by restriction enzyme analysis. The resulting plasmid, designated pBRHtrp, expresses β-lactamase, imparting ampicillin resistance, and it contains a DNA fragment including the trp promoter-operator and encoding a first protein comprising a fusion of the first six amino acids of the trp leader and approximately the last third of the trp E polypeptide (this polypeptide is designated LE'), and a second protein corresponding to approximately the first half of the trp D polypeptide (this polypeptide is designated D'), and a third protein coded for by the tetracycline resistance gene.

pBRH trp was digested with EcoRI restriction enzyme and the resulting fragment 1 isolated by PAGE and electroelution. EcoRI-digested plasmid pSom 11 (K. Itakura et al, Science 198, 1056 (1977); G.B. patent publication no. 2 007 676 A) was combined with this fragment 1. The mixture was ligated with T_4 DNA ligase as previously described and the resulting DNA transformed into E. coli K-12 strain 294 as previously described. Transformant bacteria were selected on ampicillin-containing plates. Resulting ampicillin-resistant colonies were screened by colony hybridization (M. Gruenstein et al., Proc Nat'l Acad Sci USA 72, 3951-3965 [1975]) using as a probe the trp promoter- operator-containing fragment 1 isolated from pBRHtrp, which had been radioactively labelled with P^{32}. Several colonies shown positive by colony hybridization were selected, plasmid DNA was isolated and the orientation of the inserted fragments determined by restriction analysis employing restriction enzymes BglII and BamHI in double digestion. E. coli 294 containing the plasmid designated pSOM7Δ2, which has the trp promoter-operator fragment in the desired orientation was grown in LB medium containing 10 µg/ml ampicillin. The cells were grown to

optical density 1 (at 550 nM), collected by centrifugation and resuspended in M9 media in tenfold dilution. Cells were grown for 2-3 hours, again to optical density 1, then lysed and total cellular protein analyzed by SDS (sodium dodcyl sulfate) area (15 percent) polyacrylamide gel electrophoresis (J.V. Maizel Jr. et al., Meth Viral 5, 180-246 (1971)).

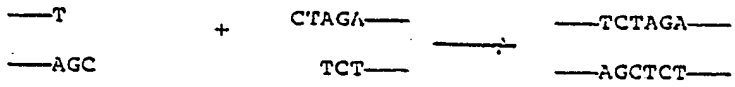
The plasmid pSom7Δ 2, 10µg, was cleaved with EcoRI and the DNA fragment 1 containing the tryptophan genetic elements was isolated by PAGE and electroelution. This fragment, 2µg, was digested with the restriction endonuclease Taq I, 2 units, 10 minutes at 37°C such that, on the average, only one of the approximately five Taq I sites in each molecule is cleaved. This partially digested mixture of fragments was separated by PAGE and an approximately 300 base pair fragment 2 that contained one EcoRI end and one Taq I end was isolated by electroelution. The corresponding Taq I site is located between the transcription start and translation start sites and is 5 nucleotides upstream from the ATG codon of the trp leader peptide. The DNA sequence about this site is shown in Figure 4. By proceeding as described, a fragment could be isolated containing all control elements of the trp operon, i.e., promoter-operator system, transcription initiation signal, and part of the trp leader ribosome binding site.

The Taq I residue at the 3' end of the resulting fragment adjacent the translation start signal for the trp leader sequence was next converted into an XbaI site. This was done by ligating the Fragment 2 obtained above to a plasmid containing a unique (i.e., only one) EcoRI site and a unique XbaI site. For this purpose, one may employ essentially any

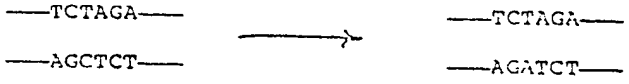
plasmid containing, in order, a replicon, a selectable marker such as antibiotic resistance, and EcoRI, XbaI and BamHI sites. Thus, for example, an XbaI site can be introduced between the EcoRI and BamHI sites of pBR322 (F. Bolivar et al., Gene 2, 95-119 [1977]) by, e.g., cleaving at the plasmid's unique Hind III site with Hind III followed by single strand-specific nuclease digestion of the resulting sticky ends, and blunt end ligation of a self annealing double-stranded synthetic nucleotide containing the recognition site such as CCTCTAGAGG. Alternatively, naturally derived DNA fragments may be employed, as was done in the present case, that contain a single XbaI site between EcoRI and BamHI cleavage residues. Thus, an EcoRI and BamHI digestion product of the viral genome of hepatitis B was obtained by conventional means and cloned into the EcoRI and BamHI sites of plasmid pGH6 (D.V. Goeddel et al., Nature 281, 544 [1979])) to form the plasmid pHS32. Plasmid pHS32 was cleaved with XbaI, phenol extracted, chloroform extracted and ethanol precipitated. It was then treated with 1 µl E. coli polymerase I, Klenow fragment (Boehringer-Mannheim) in 30 µl polymerase buffer (50 mM potassium phosphate pH 7.4, 7mM $MgCl_2$, 1 mM β-mercaptoethanol) containing 0.1mM dTTP and 0.1mM dCTP for 30 minutes at 0°C then 2 hr. at 37°C. This treatment causes 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site to be filled in:

Two nucleotides, dC and dT, were incorporated giving an end with two 5' protruding nucleotides. This linear residue of

plasmid pHS32 (after phenol and chloroform extraction and recovery in water after ethanol precipitation) was cleaved with EcoRI. The large plasmid Fragment was separated from the smaller EcoRI-XbaI fragment by PAGE and isolated after electroelution. This DNA fragment from pHS32 (0.2 µg), was ligated, under conditions similar to those described above, to the EcoRI-Taq I fragment of the tryptophan operon (0.01 µg). In this process the Taq I protruding end is ligated to the XbaI remaining protruding end even though it is not completely Watson-Crick base-paired:



A portion of this ligation reaction mixture was transformed into E. coli 294 cells as in part I. above, heat treated and plated on LB plates containing ampicillin. Twenty-four colonies were selected, grown in 3 ml LB media, and plasmid isolated. Six of these were found to have the XbaI site regenerated via E. coli catalyzed DNA repair and replication:



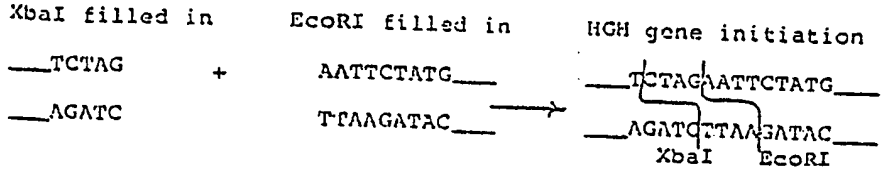
These plasmids were also found to cleave both with EcoRI and HpaI and to give the expected restriction fragments. One plasmid 14, designated pTrp 14, was used for expression of heterologous polypeptides, as next discussed.

The plasmid pHGH 107 (D.V. Goeddel et al, Nature, 281, 544, 1979) contains a gene for human growth hormone made up of 23 amino acid codons produced from synthetic DNA fragments and 163

amino acid codons obtained from complementary DNA produced via reverse transcription of human growth hormone messenger RNA. This gene, 3, though it lacks the codons of the "pre" sequence of human growth hormone, does contain an ATG translation initiation codon. The gene was isolated from 10 µg pHGH 107 after treatment with EcoRI followed by E. coli polymerase I Klenow fragment and dTTP and dATP as described above. Following phenol and chloroform extraction and ethanol precipitation the plasmid was treated with BamHI. The human growth hormone ("HGH") gene-containing fragment 3 was isolated by PAGE followed by electroelution. The resulting DNA fragment also contains the first 350 nucleotides of the tetracycline resistance structural gene, but lacks the tetracyline promoter-operator system so that, when subsequently cloned into an expression plasmid, plasmids containing the insert can be located by the restoration of tetracycline resistance. Because the EcoRI end of the fragment 3 has been filled in by the Klenow polymerase I procedure, the fragment has one blunt and one sticky end, ensuring proper orientation when later inserted into an expression plasmid.

The expression plasmid pTrp14 was next prepared to receive the HGH gene-containing fragment prepared above. Thus, pTrp14 was XbaI digested and the resulting sticky ends filled in with the Klenow polymerase I procedure employing dATP, dTTP, dGTP and dCTP. After phenol and chloroform extraction and ethanol precipitation the resulting DNA was treated with BamHI and the resulting large plasmid fragment isolated by PAGE and electroelution. The pTrp14-derived fragment had one blunt and one sticky end, permitting recombination in proper orientation with the HGH gene containing fragment 3 previously described.

The HGH gene fragment 3 and the pTrp14 Xba-BamHI fragment were combined and ligated together under conditions similar to those described above. The filled in XbaI and EcoRI ends ligated together by blunt end ligation to recreate both the XbaI and the EcoRI site:



This construction also recreates the tetracycline resistance gene. Since the plasmid pHGH 107 expresses tetracycline resistance from a promoter lying upstream from the HGH gene (the lac promoter), this construction, designated pHGH 207, permits expression of the gene for tetracycline resistance under the control of the tryptophan promoter-operator. Thus the ligation mixture was transformed into E. coli 294 and colonies selected on LB plates containing 5 µg/ml tetracycline.

Construction of pBGH33-1 (Figure 5)

The structure of pHGH207-1 which has the entire human growth hormone gene sequence is shown. The front part of this gene is synthetic as is described by Goeddel et al., Nature 281, 544 (1979). In the following a plasmid was constructed containing the BGH gene in the same orientation and in the same position with respect to the trp-promotor as is the HGH gene in pHGH 207-1.

Twenty µl (i.e. 10µg) of the plasmid DNA was digested wth Bam HI and PvuII as follows: To the twenty µl of DNA we added 5 µl 10X restriction enzyme buffer (500mM NaCl, 100 mM Tris HCl pH 7.4, 100 mM $MgSO_4$ and 10 mM DTT), 20 µl H_2O and 10 units BamHI

restriction enzyme and 2 µl PvuII restriction enzyme. Subsequently, this reaction mixture was incubated at 37°C for 90 minutes. The mixture was loaded on a 6 percent acrylamide gel and electrophoresis was carried out for 2 hours at 50 mA. The DNA in the gel was stained with Ethidium bromide and visualized with UV-light. The band corresponding to the 365 bp (with reference to a HaeIII digest of pBR322) fragment was excised and inserted in a dialysis bag and the DNA was electroeluted using a current of 100 mA. The liquid was removed from the bag and its salt concentration adjusted to 0.3M NaCl. Two volumes of ethanol were added and the DNA precipitated at -70°C. The DNA was spun down in an Eppendorf centrifuge, washed with 70 percent ethanol and dried and resuspended in 10 µl TAE (10 mM Tris HCl pH7.4, 0.1 mM EDTA). Similarly, the large XbaI Bam HI fragment of pHGH 207-1 and the XbaI, partial PvuII 570 bp fragment of pBGH33 were isolated.

Two µl of each of the thus isolated DNA fragments were mixed. 1 µl 10mM ATP and 1 µl 10x ligase buffer (200 mM Tris HCl pH7.5, 100mM $MgCl_2$, 20 mM DTT) and 1 µl T_4 DNA ligase and 2 µl H_2O were added. Ligation was done over night at 4°C. This mixture was used to transform competent E. coli K-12 294 cells as follows: 10 ml L-broth was inoculated with E. coli K-12 294 and incubated at 37°C in a shaker bath at 37°C. At OD_{550} of 0.8 the cells were harvested by spinning in a Sorvall centrifuge for 5 min. at 6000 rpm. The cell pellet was washed/resuspended in 0.15 M NaCl, and again spun. The cells were resuspended in 75 mM $CaCl_2$, 5 mM $MgCl_2$ and 10 mM Tris HCl pH7.8 and incubated on ice for at least 20 min. The cells were spun down for 5 min at 2500 rpm and resuspended in the same buffer. To 250 µl of this cell suspension each of the ligation mixtures was added and incubated for 60 min on ice. The cells were heat shocked for 90 seconds at 42°C, chilled and 2 ml L-broth was added. The cells

were allowed to recover by incubation at 37°C for 1 hour. 100 µl of this cell suspension was plated on appropriate plates which were subsequently incubated over night at 37°C. The plasmid structure in several of the colonies thus obtained is shown in Figure 5 (pBGH 33-1).

All further constructions were done using the same procedures, as described above, mutatis mutandis.

Construction of the hybrid growth hormone genes HBGH and BHGH (Figure 5)

The two PvuII sites in the HGH and BGH genes are at identical positions. Exchange of PvuII fragments is possible without changing the reading frame of the messenger RNA of these genes. The large difference in expression of both genes is due to differences in initiation of protein synthesis at the beginning of the messages. Therefore, the front part of both genes were exchanged thus constructing hybrid genes that upon transcription would give hybrid messenger RNAs. The two plasmids, pBHGH and pHBGH, were constructed as follows:

From pHGH207-1 there were isolated the large BamHI-XbaI fragment and the 857 bp BamHI (partial) PvuII fragment containing the HGH gene without its front part. From pBGH33-1 there was isolated the 75 bp XbaI-PvuII fragment that contains the front part of the BGH gene. After ligation and transformation pBHGH was obtained. pHBGH was constructed in a similar way to pBHGH; in this case the back part was derived from pBGH33-1 whereas the front part, the 75 bp XbaI-PvuII fragment, was derived from pHGH207-1.

Design and cloning of the synthetic front part of the BGH gene (Figure 6)

The DNA sequence up to the PvuII site of the BGH and HGH genes codes for 22 amino acids. Since the front part of the HGH gene had excellent protein synthesis initiation properties, the sequence of the front part of BGH was designed such that the number of nucleotide changes in the BGH gene would be minimal with respect to the HGH gene. Only 14 base pair changes from the natural BGH sequence were made in order to code for the proper BGH amino acid sequence and reduce conformational structure in the prospective mRNA. The DNA sequence is shown in Figure 6. The sequence ends with EcoRI and HindIII sticky ends to make cloning in a vector easy. Close to the HindIII site is a PvuII site for the proper junction with the remaining part of the BGH gene.

The fragments U1 to U6 and L1 to L6 were synthesized chemically according to the procedures described above. All the fragments except U1 and L6 were mixed and kinased. After addition of U1 and L6 the mixed fragments were ligated, purified on a 6 percent polyacrylamide gel and the 75 bp band extracted and isolated according to standard procedures. This fragment was inserted into pBR322 that had been cut with EcoRI and HindIII. Thus plasmid pBR322-01 was obtained.

Replacement of the natural front part of the BGH gene by the synthetic front part. (Figure 7)

From pBR322-01 the cloned synthetic front of the BGH gene was excised with EcoRI and PvuII, and the resulting 70 bp fragment was isolated. From pBGH33-1 the large EcoRI-BamHI fragment and the 875 bp BamHI (partial) PvuII fragment was isolated. The three

fragments were isolated and ligated and used to transform E. coli K-12 294 as described before. Thus, pBGH33-2 was obtained. This plasmid contains the entire BGH gene but does not have a promotor. Therefore, pBGH33-2 was cut with EcoRI and the trp-promotor containing 310 bp EcoRI fragment derived from pHGH207-1 was inserted by ligation. After transformation tetracycline resistant colonies were analyzed. Therefore, these colonies had the inserted trp-promotor oriented towards the HGH- and tet-gene as shown in the figure.

Repair of the 3'-end of the BGH gene. (Figure 8)

The sequences beyond the second PvuII site of the BGH gene are derived from the HGH gene. One of the amino acids at the end is different from that in the natural BGH gene. This 3'-end was repaired as follows. A synthetic DNA fragment as shown was synthesized. It is flanked by an EcoRI and a HindIII end to facilitate cloning and contains a PvuII site and 3 amino acid codons and a stop codon in the reading frame of the BGH gene itself. This fragment was inserted into EcoRI-HindIII opened pBR322. Thus pBR322-02 was obtained. Subsequently this plasmid was cut with PvuII and BamHI and the 360 bp fragment was isolated. From pBGH33-3, which has the entire BGH gene with the synthetic front part, the large BamHI and XbaI fragment and the 570 bp XbaI (partial) PvuII fragment was isolated. These three fragments were ligated and used to transform cells. Thus, pBGH33-4 was obtained. In this plasmid a unique HindIII site is present between the stop codon of the BGH gene and the start codon of the tet-mRNA. Both genes are transcribed under direction of the trp promotor.

A typical growth medium used to derepress and produce high levels of BGH per liter (Figure 9) contains: 5.0 g $(NH_4)_2SO_4$, 6.0 g K_2HPO_4, 3.0 g $NaH_2PO_4.2H_2O$, 1.0 g sodium citrate, 2.5 g glucose, 5 mg tetracycline, 70 mg thiamine HCl, and 60 g $MgSO_4.7H_2O$.

While the present invention has been described, in its preferred embodiments, with reference to the use of E. coli transformants, it will be appreciated that other microorganisms can be employed mutatis mutandis. Examples of such are other E. coli organisms, e.g. E. coli B., E. coli W3110 ATCC No. 31622 (F^-, λ^-, gal^-, prototroph), E. coli x 1776, ATCC No. 31537, E. coli D1210, E. coli RV308, ATCC No. 31608, etc., Bacillus subtilis strains, Pseudomonas strains, etc. and various yeasts, e.g., Saccharomyces cerevisiae many of which are deposited and (potentially) available from recognized depository institutions e.g., ATCC. Following the practice of this invention and the final expression of intended polypeptide product, extraction and purification techniques may be those customarily employed in this art, known per se.

Claims

1. A method of constructing a DNA sequence for a messenger RNA encoding a polypeptide comprising a functional protein or a bioactive portion thereof, said DNA sequence being designed for insertion together with appropriately positioned translational start and stop signals into a microbial expression vector under the control of a microbially operable promotor, the method comprising:

a) providing a fragment of said DNA sequence encoding a C-terminal portion of said polypeptide, said fragment encoding a polypeptide conforming in sequence to the natural sequence of this polypeptide,

b) providing a fragment of said DNA sequence encoding the N-terminal portion of said polypeptide, and

c) ligating the fragments of steps a) and b) in proper reading frame relation to one another,

said fragment of step b) being characterized in that the nucleotides thereof are sequentially arranged so as to provide, on transcription, corresponding messenger RNA that

1) properly encodes the respective portion of the amino acid sequence of said polypeptide and

2) demonstrates levels of conformational structure insufficient to interfere with its accessibility for efficient ribosomal translation.

said DNA sequence being exclusive of the hybrid DNA sequence of human growth hormone set forth in Table 1 of the specification hereof.

2. The method according to Claim 1 wherein the first nucleotide of said fragment of step b) corresponds to nucleotide +1 of the corresponding messenger RNA.

3. The method according to Claim 1 wherein the first nucleotide of said fragment of step b) corresponds to a nucleotide of the translational start signal.

4. The method according to Claim 1 wherein said fragment of step b) extends from the nucleotide corresponding to a nucleotide of the translational start signal through at least the nucleotide representing the last of about the proximal 25 amino acids of said polypeptide.

5. The method according to Claim 1 wherein said fragment of step b) extends from nucleotide +1 to about nucleotide +100 of the corresponding messenger RNA.

6. The method according to Claim 1 wherein the DNA sequence of said fragment of step b) is as depicted in Figure 1 as BGH synthetic.

7. The method according to any preceding claim wherein said DNA sequence is inserted together with appropriately positioned translational start and stop signals into a microbial expression vector and is therein brought under the control of a microbially operable promotor, to provide the corresponding microbial expression vehicle.

8. The method according to Claim 7 wherein a microorganism is transformed with said microbial expression vehicle to provide the corresponding transformed microorganism.

9. The method according to Claim 8 wherein the resulting transformed microorganism is grown under suitable fermentation conditions and caused to produce said polypeptide, said polypeptide being subsequently recovered from the fermentation medium.

10. The method of Claims 8 or 9 wherein said microorganism is an E. coli strain and said expression vector is an E. coli plasmid.

11. A plasmid selected from pBR 322-01, pBGH 33-3 and pBGH 33-4.

12. A transformant microorganism harboring one of the plasmids according to Claim 11.

13. A culture of a microorganism according to Claim 12.

A composition of matter comprising bovine growth hormone essentially free of other proteins of bovine origin.

14. A microorganism capable of producing bovine growth hormone in amounts exceeding about 100 thousand copies per cell.

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hGH natural	amino acids:	Met Phe Pro Ala Met Ser Leu Ser Gly Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His Gln
	bases:	ATG TTC CCA GCC ATG TCC TTG TCC GGC CTG TTT GCC AAC GCT GTG CTC CGG GCT CAG CAC CTG CAT CAG
		PvuII
hGH synthetic	amino acids:	Met Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln
	bases:	ATG TTC CCA ACT ATA CCA CTA TCT CGT CTA TTC GAT AAC GCT ATG CTT CGT GCT CAT CGT CTT CAT CAG
		PvuII
hGH synthetic	amino acids:	Met Phe Pro Ala Met Ser Leu Ser Gly Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His Gln
	bases:	ATG TTC CCA GCT ATG TCT CTA TCT GGT CTA TTC GCT AAC GCT GTT CTT CGT GCT CAG CAT CTT CAT CAG
		PvuII

FIG. 1.

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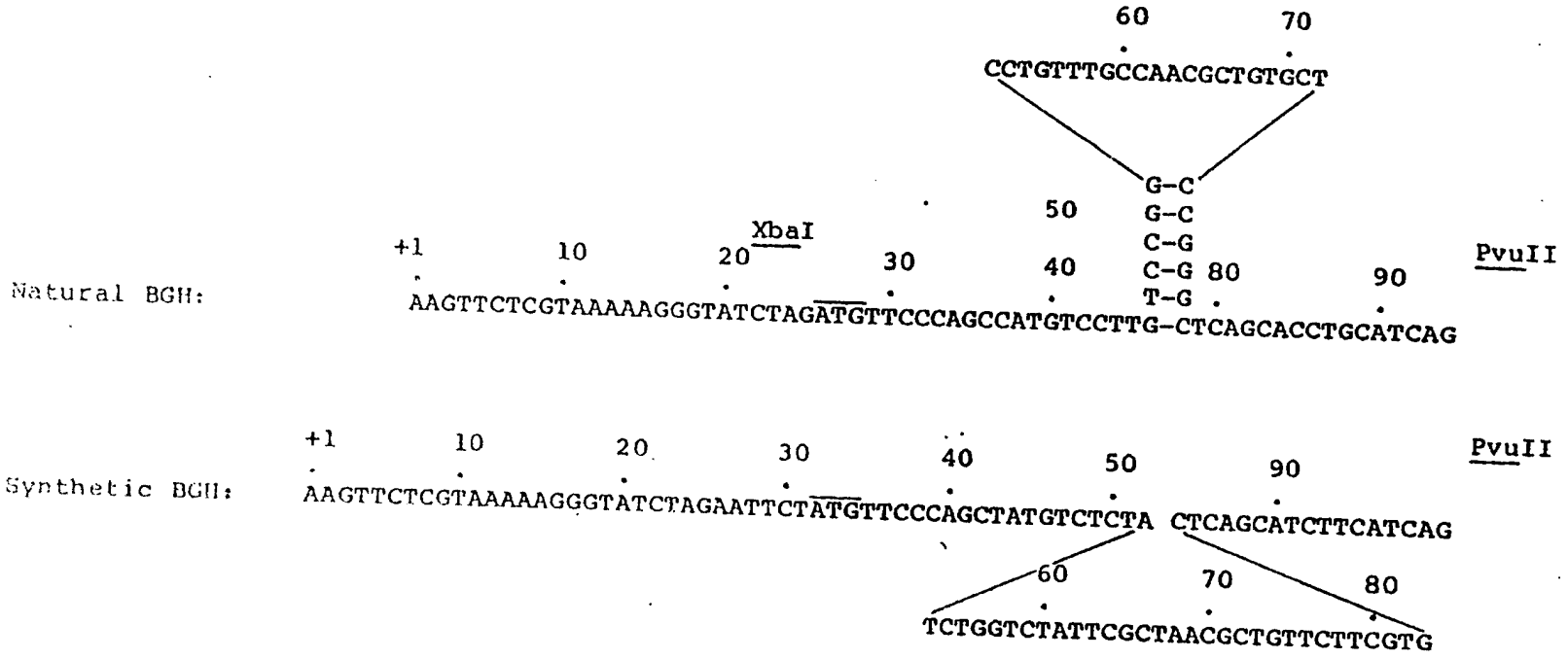


FIG. 2.

Natural BGH				Synthetic BGH			
5'	3'	length	kcal/mol	5'	3'	length	kcal/mol
14	45	8	-11.80	14	79	7	-5.50
16	31	6	4.00	16	37	6	-4.00
33	101	6	-15.40	38	104	6	-15.40
46	78	6	-15.20	52	84	-	>-10

FIG. 3.

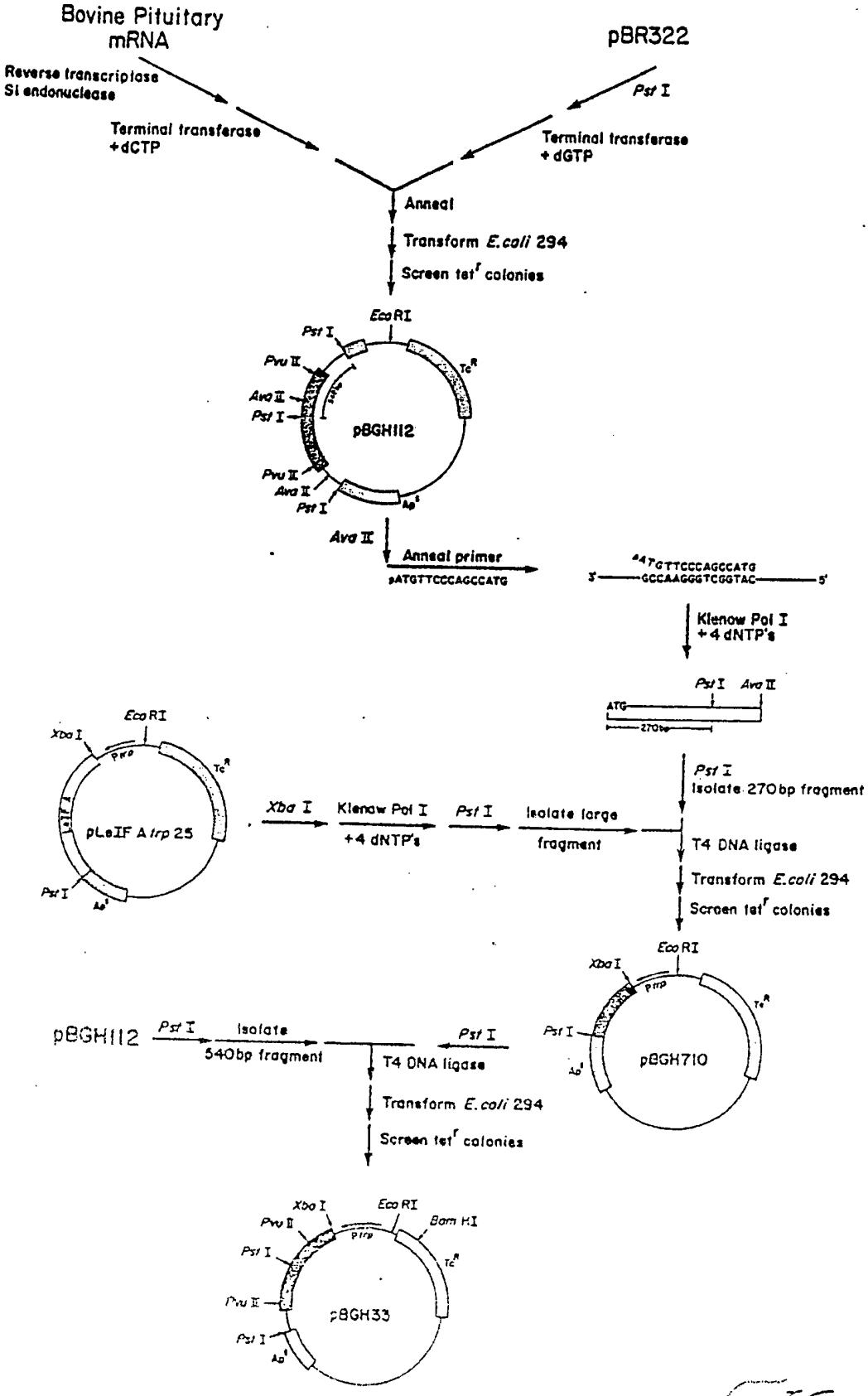

Bovine Pituitary mRNA
Reverse transcriptase
S1 endonuclease
Terminal transferase
+dCTP
pBR322
Pst I
Terminal transferase
+dGTP
Anneal
Transform E.coli 294
Screen tet^r colonies
pBGH112
Ava II
Anneal primer
pATGTTCCCAGCCATG
Klenow Pol I
+4 dNTP's
Pst I
Isolate 270bp fragment
pLeIF A trp 25
Xba I
Klenow Pol I
+4 dNTP's
Pst I
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
pBGH710
pBGH112
Isolate
540bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
pBGH33

FIG. 4.

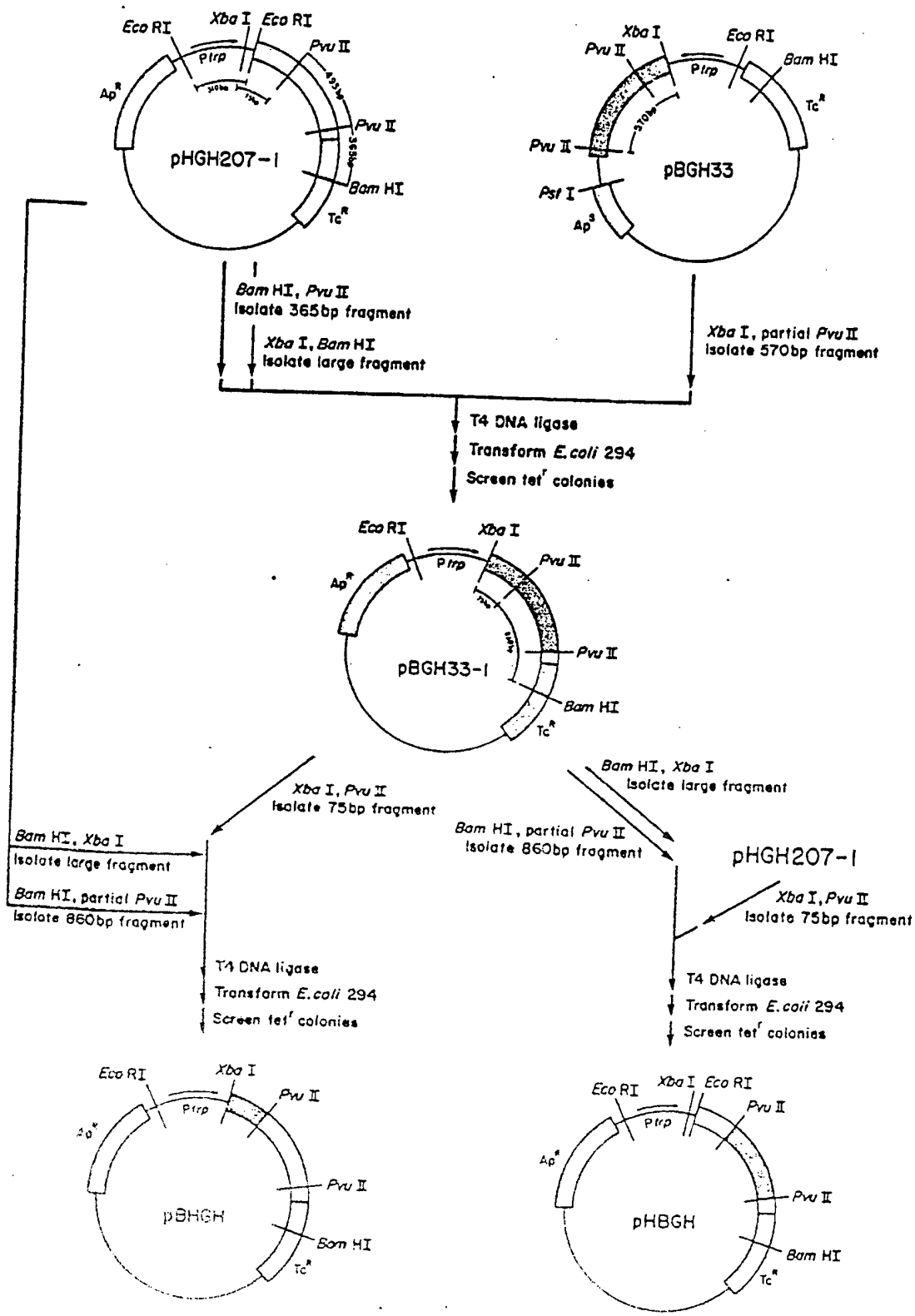
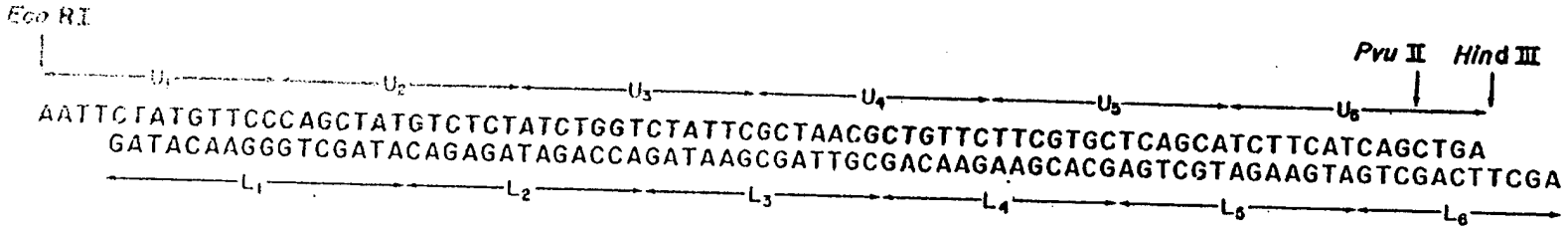

Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pHGH207-1
Pvu II
Bam HI
Tc^R
Xba I
Pvu II
Eco RI
Bam HI
Ptrp
Tc^R
570bp
Pvu II
pBGH33
Pst I
Ap^R
Bam HI, Pvu II
Isolate 365bp fragment
Xba I, Bam HI
Isolate large fragment
Xba I, partial Pvu II
Isolate 570bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Ptrp
Pvu II
Ap^R
pBGH33-1
Pvu II
Bam HI
Tc^R
Xba I, Pvu II
Isolate 75bp fragment
Bam HI, Xba I
Isolate large fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
Bam HI, Xba I
Isolate large fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
pHGH207-1
Xba I, Pvu II
Isolate 75bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Pvu II
Ptrp
Ap^R
pBHGH
Pvu II
Bam HI
Tc^R
Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pHBGH
Pvu II
Bam HI
Tc^R

FIG. 5



Mix Synthetic Oligonucleotides

$U_2-U_3-U_4-U_5-U_6$

$L_1-L_2-L_3-L_4-L_5$

T4 polynucleotide kinase

Phenol extract
Ethanol precipitate

Add U_1 and L_6
T4 DNA ligase

pBR322

15% polyacrylamide gel

Eco RI, *Hind* III
Isolate large fragment

Isolate 75bp band

T4 DNA ligase

Transform *E.coli* 294

Screen amp^r tet^s colonies

pBR322-01

FIG. 6.

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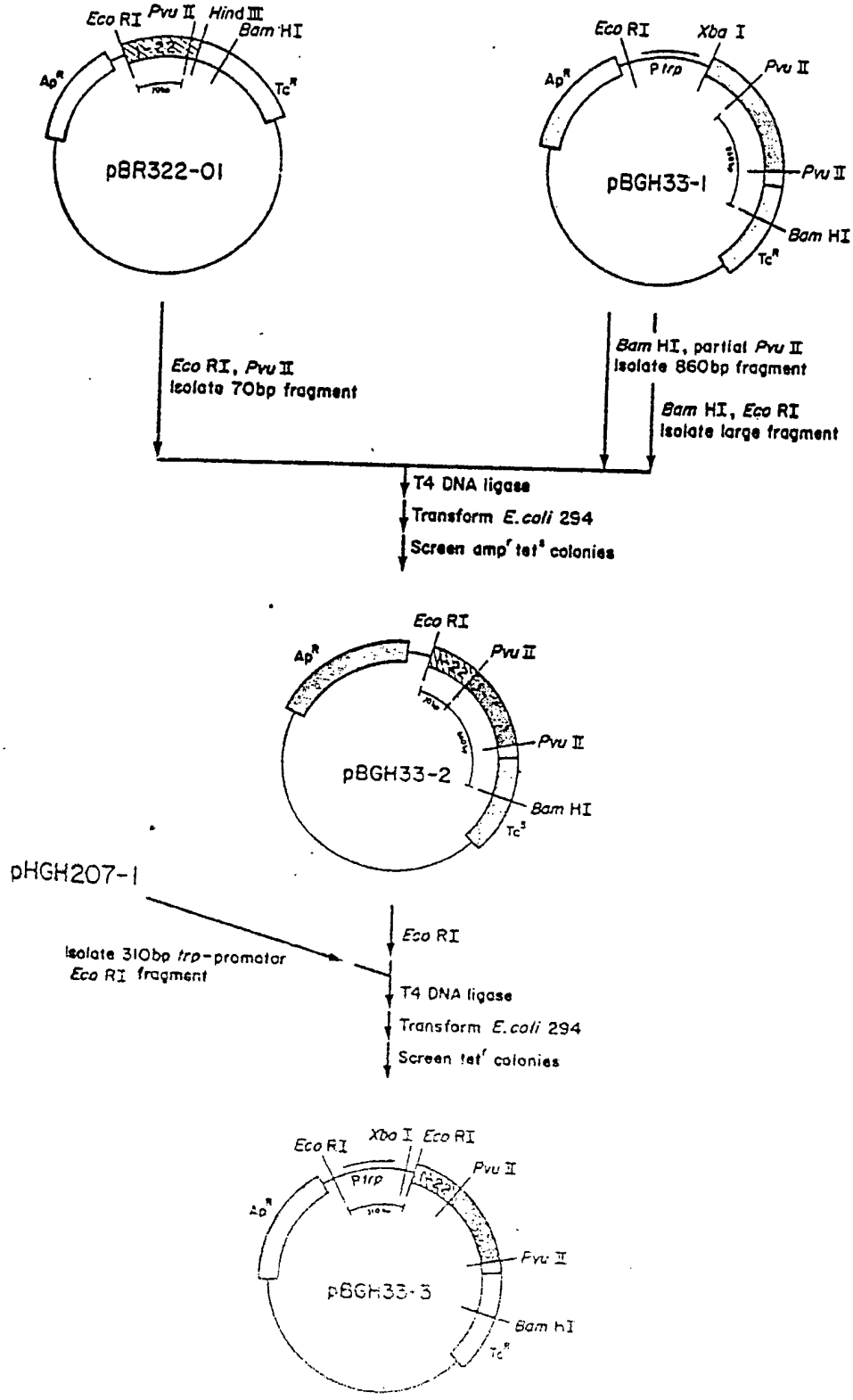

Eco RI
Pvu II
Hind III
Bam HI
Ap^R
Tc^R
pBR322-01
Eco RI
Xba I
P trp
Pvu II
pBGH33-1
Pvu II
Bam HI
Tc^R
Eco RI, Pvu II
Isolate 70bp fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
Bam HI, Eco RI
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Screen amp^r tet^s colonies
Eco RI
Ap^R
Pvu II
pBGH33-2
Pvu II
Bam HI
Tc^S
pHGH207-1
Isolate 310bp trp-promotor
Eco RI fragment
Eco RI
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Eco RI
P trp
Pvu II
Ap^R
pBGH33-3
Pvu II
Bam HI
Tc^R

FIG. 7.

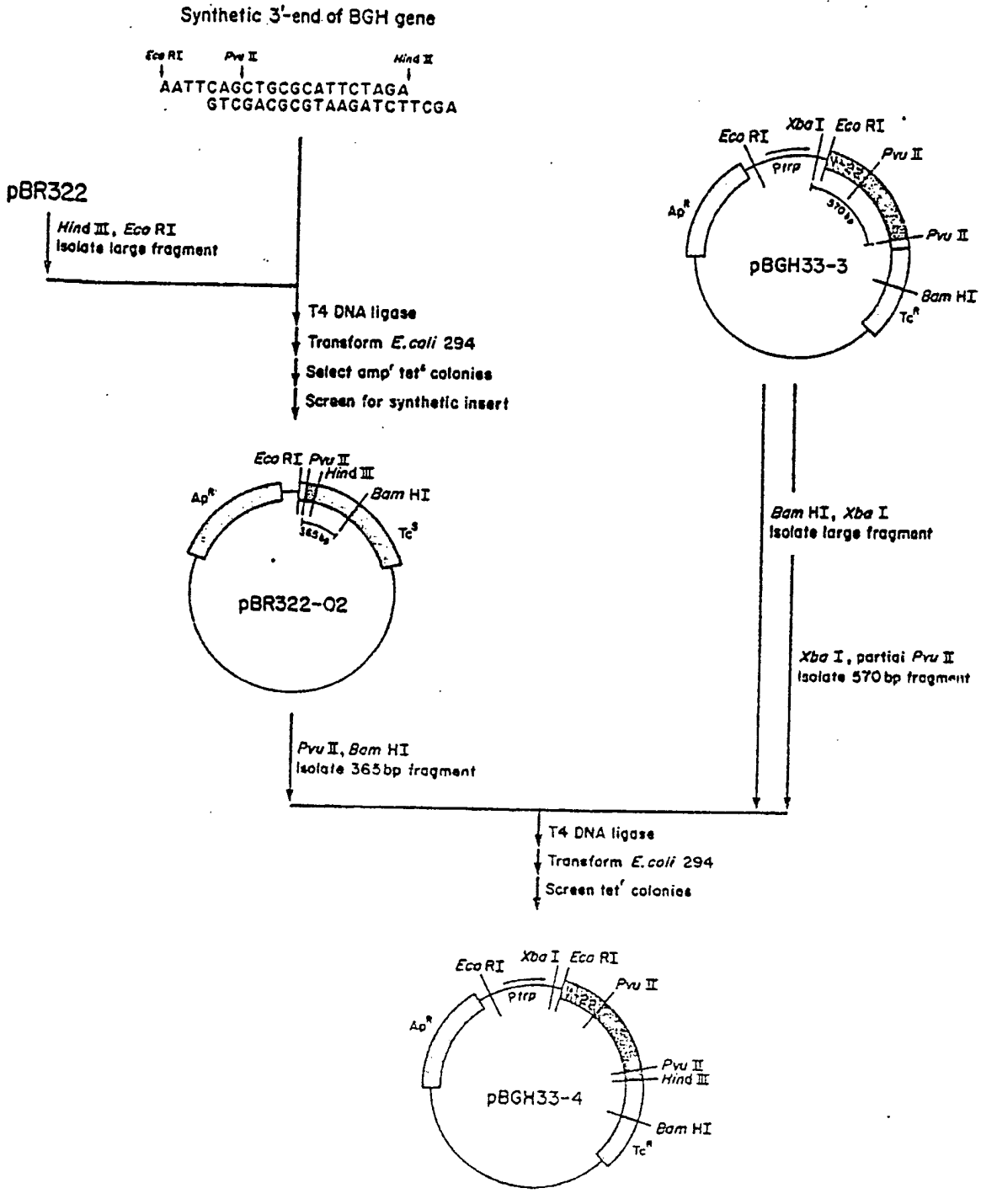
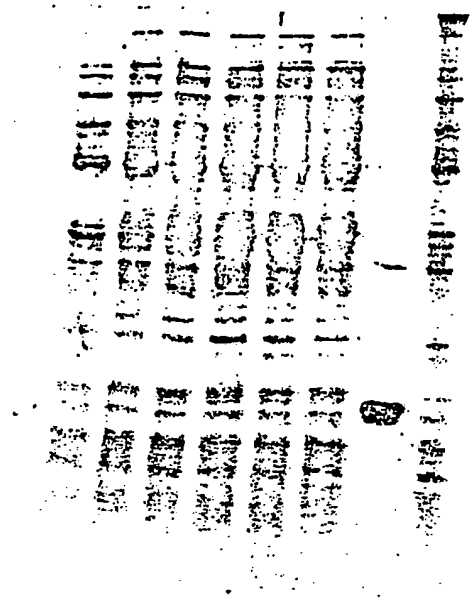

Synthetic 3'-end of BGH gene
Eco RI
Pvu II
Hind III
AATTCAGCTGCGCATTCTAGA
GTCGACGCGTAAGATCTTCGA
pBR322
Hind III, Eco RI
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Select amp^r tet^s colonies
Screen for synthetic insert
Eco RI
Pvu II
Hind III
Bam HI
Ap^R
Tc^S
365bp
pBR322-02
Pvu II, Bam HI
Isolate 365bp fragment
Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pBGH33-3
Bam HI
Tc^R
Bam HI, Xba I
Isolate large fragment
Xba I, partial Pvu II
Isolate 570bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pBGH33-4
Pvu II
Hind III
Bam HI
Tc^R

FIG. 8.

A

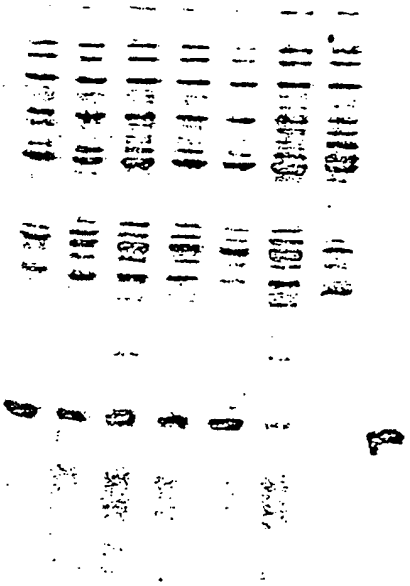


1.1 2.1 4.1 7.3 9.8 15.8 (+) (−)

bGH

GENENTECH hGH

B



hGH bGH bGH #1 bGH #2 (+) hGH (−) (−) hGH

FIG. 9.

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EP-A- 0 022 242
GB-A- 2 073 245

PROC. NATL. ACAD. SCI, USA, vol. 74, no. 10, October 1977, pages 4163-4167; D.A. STEEGE: "5'-Terminal nucleotide sequence of Escherichia coli lactose repressor mRNA: features of translational initiation and reinitiation sites"

CHEMICAL ABSTRACTS, vol. 92, no. 23, 9th June 1980, page 201, no. 192883j, Columbus, Ohio, US; D. ISERENTANT et al.: "Secondary structure of mRNA and efficiency of translation initiation" & GENE 1980, 9(1-2), 1-12,

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EP 0 075 444 B1

Description

The present invention provides methods and means for preparing DNA sequences that provide messenger RNA having improved translation characteristics. The resulting messenger RNA may be highly efficient in translation to give substantial amounts of polypeptide product that is normally heterologous to the host microorganism. The DNA sequences which are ultimately expressed, that is, transcribed into messenger RNA (mRNA) which is in turn translated into polypeptide product, are, in essential part, synthetically prepared, in accordance with this invention, utilizing means that favor the substantial reduction or elimination of secondary and/or tertiary structure in the corresponding transcribed mRNA. An absence or substantial reduction in such secondary/tertiary structure involving the 5' end of mRNA permits effective recognition and binding of ribosomes(s) to the mRNA for subsequent translation. Thus, the efficiency of translation is not hindered or impaired by conformational impediments in the structure of the transcribed mRNA. Methods and means for measuring mRNA secondary/tertiary structure are also described as well as associated means designed to insure that secondary/tertiary structure is kept below certain preferred limits. This invention is exemplified by the preparation of various preferred protein products.

With the advent of recombinant DNA technology, the controlled microbial production of an enormous variety of useful polypeptides has become possible, putting within reach the microbially directed manufacture of hormones, enzymes, antibodies, and vaccines useful against a wide variety of diseases. Many mammalian polypeptides, such as human growth hormone and leukocyte interferons, have already been produced by various microorganisms.

One basic element of recombinant DNA technology is the plasmid, an extrachromosomal loop of double-stranded DNA found in bacteria oftentimes in multiple copies per cell. Included in the information encoded in the plasmid DNA is that required to reproduce the plasmid in daughter cells (i.e., a "replicon") and ordinarily, one or more selection characteristics, such as resistance to antibiotics, which permit clones of the host cell containing the plasmid of interest to be recognized and preferentially grown in selective media. The utility of such bacterial plasmids lies in the fact that they can be specifically cleaved by one or another restriction endonuclease or "restriction enzyme", each of which recognizes a different site on the plasmidic DNA. Heterologous genes or gene fragments may be inserted into the plasmid by endwise joining at the cleavage site or at reconstructed ends adjacent to the cleavage site. (As used herein, the term "heterologous" refers to a gene not ordinarily found in, or a polypeptide sequence ordinarily not produced by, a given microorganism, whereas the term "homologous" refers to a gene or polypeptide which is found in, or produced by the corresponding wild-type microorganism.) Thus formed are so-called replicable expression vehicles.

DNA recombination is performed outside the microorganism, and the resulting "recombinant" plasmid can be introduced into microorganisms by a process known as transformation and large quantities of the heterologous gene-containing recombinant plasmid are obtained by growing the transformant. Moreover, where the gene is properly inserted with reference to portions of the plasmid which govern the transcription and translation of the encoding DNA, the resulting plasmid can be used to actually produce the polypeptide sequence for which the inserted gene codes, a process referred to as expression. Plasmids which express a (heterologous) gene are referred to as replicable expression vehicles.

Expression is initiated in a DNA region known as the promotor. In some cases, as in the lac and trp systems discussed infra, promotor regions are overlapped by "operator" regions to form a combined promotor-operator. Operators are DNA sequences which are recognized by so-called repressor proteins which serve to regulate the frequency of transcription initiation from a particular promoter. In the trancription phase of expression, RNA polymerase recognizes certain sequences in and binds to the promoter DNA. The binding interaction causes an unwinding of the DNA in this region, exposing the DNA as a template for synthesis of messenger RNA. The messenger RNA serves as a template for ribosomes which bind to the messenger RNA and translate the mRNA into a polypeptide chain having the amino acid sequence for which the RNA/DNA codes. Each amino acid is encoded by a nucleotide triplet or "codon" which collectively make up the "structural gene", i.e., that part of the DNA sequence which encodes the amino acid sequence of the expressed polypeptide product.

After binding to the promoter, RNA polymerase initiates the transcription of DNA encoding a ribosome binding site including a translation initiation or "start" signal (ordinarily ATG, which in the resulting messenger RNA becomes AUG), followed by DNA sequences encoding the structural gene itself. So-called translational stop codons are transcribed at the end of the structural gene whereafter the polymerase may form an additional sequence of messenger RNA which, because of the presence of the translational stop signal, will remain untranslated by the ribosomes.

Ribosomes bind to the binding site provided on the messenger RNA, in bacteria ordinarily as the mRNA is

being formed, and direct subsequently the production of the encoded polypeptide, beginning at the translation start signal and ending at the previously mentioned stop signal(s). The resulting product may be obtained by lysing the host cell and recovering the product by appropriate purification from other bacterial proteins.

Polypeptides expressed through the use of recombinant DNA technology may be entirely heterologous, functional proteins, as in the case of the direct expression of human growth hormone, or alternatively may comprise a bioactive heterologous polypeptide portion and, fused thereto, a portion of the amino acid sequence of a homologous polypeptide, as in the case of the production of intermediates for somatostatin and the components of human insulin. In the latter cases, for example, the fused homologous polypeptide comprised a portion of the amino acid sequence for beta galactosidase. In those cases, the intended bioactive product is rendered bioinactive within the fused, homologous/ heterologous polypeptide until it is cleaved in an extracellular environment. Fusion proteins like those just mentioned can be designed so as to permit highly specific cleavage of the precusor protein from the intended product, as by the action of cyanogen bromide on methionine, or alternatively by enzymatic cleavage. See, eg., G.B. Patent Publication No. 2 007 676 A.

If recombinant DNA technology is to fully sustain its promise, systems must be devised which optimize expression of gene inserts, so that the intended polypeptide products can be made available in controlled environments and in high yields.

Promoter Systems

As examples, the beta lactamase and lactose promoter systems have been advantageously used to initiate and sustain microbial production of heterologous polypeptides. Details relating to the make-up and construction of these promoter systems have been published by Chang et al., Nature 275, 617 (1978) and Itakura et al., Science 198, 1056 (1977), which are hereby incorporated by reference. More recently, a system based upon tryptophan, the so-called trp promoter system, has been developed. Details relating to the make-up and construction of this system have been published by Goeddel et al., Nucleic Acids Research 8, 4057 (1980) and Kleid et al., U.S.S.N. 133, 296, filed March 24, 1980, (or the equivalent European Patent Publication 0036776) which are hereby incorporated by reference. Numerous other microbial promoters have been discovered and utilized and details concerning their nucleotide sequences, enabling a skilled worker to ligate them functionally within plasmid vectors, have been published -- see, e.g. Siebenlist et al., Cell 20, 269 (1980), which is incorporated herein by this reference.

Historically, recombinant cloning vehicles (extrachromosomal duplex DNA having, inter alia., a functional origin of replication) have been prepared and used to transform microorganisms -- cf. Ullrich et al., Science 196, 1313 (1977). Later, there were attempts to actually express DNA gene inserts encoding a heterologous polypeptide. Itakura et al. (Science 198, 1056 (1977)) expressed the gene encoding somatostatin in E. coli. Other like successes followed, the gene inserts being constructed by organic synthesis using newly refined technology. In order, among other things, to avoid possible proteolytic degradation of the polypeptide product within the microbe, the genes were ligated to DNA sequences coding for a precursor polypeptide. Extracellular cleavage yielded the intended protein product, as discussed above.

In the case of larger proteins, chemical synthesis of the underlying DNA sequence proved unwieldy. Accordingly, resort was had to the preparation of gene sequences by reverse transcription from corresponding messenger RNA obtained from requisite tissues and/or culture cells. These methods did not always prove satisfactory owing to the termination of transcription short of the entire sequence; and/or the desired sequence would be accompanied by naturally occurring precursor lender or signal DNA. Thus, these attempts often have resulted in incomplete protein product and/or protein product in non-cleavable conjugate form -- cf. Villa-Komaroff et al., Proc. Natl. Acad. Sci. (USA) 75, 3727 (1978) and Seeburg et al., Nature 276, 795 (1978).

In order to avoid these difficulties, Goeddel et al., Nature 281, 544 (1979), constructed DNA, inter alia encoding human growth hormone, using chemically synthesized DNA in conjunction with enzymatically synthesized DNA. This discovery thus made available the means enabling the microbial expression of hybrid DNA (combination of chemically synthesized DNA with enzymatically synthesized DNA), notably coding for proteins of limited availability which would probably otherwise not have been produced economically. The hybrid DNA (encoding heterologous polypeptide) is provided in substantial portion, preferably a majority, via reverse transcription of mRNA,while the remainder is provided via chemical synthesis. In a preferred embodiment, synthetic DNA encoding the first 24 amino acids of human growth hormone (HGH) was constructed according to a plan which incorporated an endonuclease restriction site in the DNA corresponding to HGH amino acids 23 and 24. This was done to facilitate a connection with

downstream HGH cDNA sequences. The various 12 oligonucleotide long fragments making up the synthetic part of the DNA were chosen following then known criteria for gene synthesis: avoidance of undue complementarity of the fragments, one with another, except, of course, those destined to occupy opposing sections of the double stranded sequence; avoidance of AT rich regions to minimize transcription termination; and choice of "microbially preferred codons." Following synthesis, the fragments were permitted to effect complementary hydrogen bonding and were ligated according to methods known per se. This work is decribed in published British Patent Specification 2055382 A, which corresponds to Goeddel et al., U.S.S.N. 55126, filed July 5, 1979 which is hereby incorporated by this reference.

While the successful preparation and expression of such hybrid DNA provided a useful means for preparing heterologous polypeptides, it did not address the general problem that eucaryotic genes are not always recognized by procaryotic expression machinery in a way which provides copious amounts of end product. Evolution has incorporated sophistication unique to discrete organisms. Bearing in mind that the eucaryotic gene insert is heterologous to the procarytic organism, the relative inefficiency in expression often observed can be true for any gene insert whether it is produced chemically, from cDNA or as a hybrid. Thus, the criteria used to construct the synthetic part of the gene for HGH, defined above, are not the sole factors influencing expression levels. For example, concentrating on codon choice as the previous workers have done--cf. British Patent Specification 2007676 A -- has not been completely successful in raising the efficiency of expression towards maximal expression levels.

Guarante et al., Science 209, 1428 (1980) experimented with several hybrid ribosome binding sites, designed to match the number of base pairs between the Shine-Dalgarno sequence and the ATG of some known E. coli binding sites, their work suggesting that the reason(s) for observed relatively low efficiencies of eucaryotic gene expression by procaryote organisms is more subtle.

That the initiation of mRNA translation may be a multicomponent process is illustrated by work reported by Iserentant and Fiers, Gene 9, 1 (1980). They postulate that secondary structure of mRNA is one of the components influencing translation efficiency and imply that the initiation codon and ribosome interaction site of secondary structured, folded mRNA must be "accessible." However, what those workers apparently mean by "accessible" is that the codon and site referred to be located on the loop, rather than the stem, of the secondary structure models they have hypothesized. Shine et al, Nature, 285 (1980), 456 and Bahramian, J. theor, Biol, both emphasise the seeming importance of secondary structure in mRNA to achieve efficient translation.

The present invention is based upon the discovery that the presence of secondary/tertiary conformational structure in the mRNA interferes with the initiation and maintenance of ribosomal binding during the translation phase of heterologous gene expression.

The present invention, relating to these findings, uniquely provides methods and means for providing efficient expression of heterologous gene inserts by the requisite microbial host. The present invention is further directed to a method of microbially producing heterologous polypeptides, utilizing specifically tailored heterologous gene inserts in microbial expression vehicles, as well as associated means. It is particularly directed to the use of synthetically derived gene insert portions that are prepared so as to both encode the desired polypeptide product and provide mRNA that has minimal secondary/ tertiary structure and hence is accessible for efficient ribosomal translation.

In preferred embodiments of the present invention, synthetic DNA is provided for a substantial portion of the initial coding sequence of a heterologous gene insert, and optionally, upstream therefrom through the ATG translational start codon and ribosome binding site. The critical portion of DNA is chemically synthesized, keeping in mind two factors: 1) the creation of a sequence that codes for the initial (N-terminal) amino acid sequence of a polypeptide comprising a functional protein or bioactive portion thereof and 2) the assurance that said sequence provides, on transcription, messenger RNA that has a secondary/tertiary conformational structure which is insufficient to interfere with its accessibility for efficient ribosomal translation, as herein defined. Such chemical synthesis may use standard organic synthesis using modified mononucleotides as building blocks such as according to the method of Crea et al., Nucleic Acids Research 8, 2331 (1980) and/or the use of site directed mutagenesis of DNA fragments such as according to the method of Razin et al., Proc. Natl. Acad Sci (USA) 75, 4268 (1978) and/or synthetic primers on certain appropriately sequenced DNA fragments followed by specific cleavage of the desired region.

The present invention is directed to a process of preparing DNA sequences comprising nucleotides arranged sequentially so as to encode the proper amino acid sequence of a given polypeptide.

This method may involve obtaining a substantial portion of the DNA coding sequence of a given polypeptide via means other than chemical synthesis, most often by reverse transcription from requisite tissue and/or culture cell messenger RNA. This fragment encodes the C-terminal portion of the polypeptide and is ligated, in accordance herewith, to a remainder of the coding sequence, e.g. obtained by chemical

synthesis, optionally including properly positioned translational start and stop signals and upstream DNA through the ribosome binding site and the first nucleotide (+1) of the resultant messenger RNA. The synthetic fragment is designed by nucleotide choice dependent on conformation of the corresponding messenger RNA according to the criteria as herein discussed.

The thus prepared DNA sequences are suited for insertion and use in replicable expression vehicles designed to direct the production of the heterologous polypeptide in a transformant microorganism. In these vehicles, the DNA sequence is operably linked to promotor systems which control its expression. The invention is further directed to the replicable expression vehicles and the transformant microorganisms so produced as well as to cultures of these microorganisms in fermentation media. This invention is further directed to associated methods and means and to specific embodiments for the directed production of messenger RNA transcripts that are accessible for efficient ribosomal translation.

Excluded from the present invention, for example, is the hybrid DNA encoding human growth hormone (HGH) as disclosed by Goeddel et al., Nature 281, 544 (1979). While this particular hybrid DNA was successfully expressed to produce the intended product, the concept of the present invention was not appreciated by these workers (and hence not taught by them) and consequently was not practised in the fortuitous preparation of their expressible hybrid DNA for HGH. This hybrid DNA has the following sequence (Table 1):

Table I

```
     1
met phe pro thr ile pro leu ser arg leu phe asp asn ala met
ATG TTC CCA ACT ATA CCA CTA TCT CGT CTA TTC GAT AAC GCT ATG

                        20
leu arg ala his arg leu his gln leu ala phe asp thr tyr gln
CTT CGT GCT CAT CGT CTT CAT CAG CTG GCC TTT GAC ACC TAC CAG

                                                40
glu phe glu glu ala tyr ile pro lys glu gln lys tyr ser phe
GAG TTT GAA GAA GCC TAT ATC CCA AAG GAA CAG AAG TAT TCA TTC

leu gln asn pro gln thr ser leu cys phe ser glu ser ile pro
CTG CAG AAC CCC CAG ACC TCC CTC TGT TTC TCA GAG TCT ATT CCG

 60
thr pro ser asn arg glu glu thr gln gln lys ser asn leu glu
ACA CCC TCC AAC AGG GAG GAA ACA CAA CAG AAA TCC AAC CTA GAG

                        80
leu leu arg ile ser leu leu leu ile gln ser trp leu glu pro
CTG CTC CGC ATC TCC CTG CTG CTC ATC CAG TCG TGG CTG GAG CCC

                                                100
val gln phe leu arg ser val phe ala asn ser leu val tyr gly
GTG CAG TTC CTC AGG AGT GTC TTC GCC AAC AGC CTA GTG TAC GGC

ala ser asp ser asn val tyr asp leu leu lys asp leu glu glu
GCC TCT GAC AGC AAC GTC TAT GAC CTC CTA AAG GAC CTA GAG GAA

120
gly ile gln thr leu met gly arg leu glu asp gly ser pro arg
GGC ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCC CGG

                        140
thr gly gln ile phe lys gln thr tyr ser lys phe asp thr asn
ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC AAG TTC GAC ACA AAC

                                                160
ser his asn asp asp ala leu leu lys asn tyr gly leu leu tyr
TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC GGG CTG CTC TAC

cys phe arg lys asp met asp lys val glu thr phe leu arg ile
TGC TTC AGG AAG GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC

180                                                 191
val gln cys arg ser val glu gly ser cys gly phe stop
GTG CAG TGC CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG
```

The chemically synthetic DNA sequences hereof extend preferably from the ATG translation initiation site, and optionally upstream therefrom a given distance, to or beyond the transcription initiation site (labelled +1 by convention), and to sequences downstream encoding a substantial part of the desired polypeptide. By way of preference, the synthetic DNA comprises upwards of approximately 75 or more nucleotide pairs of the structural gene representing about the proximal (N-terminal) 25 amino acids of the intended polypeptide. In particularly preferred embodiments, the synthetic DNA sequence extends from about the translation initiation site (ATG) to about nucleotide 75 of the heterologous gene. In alternative terms, the synthetic DNA sequence comprises nucleotide pairs from +1 (transcription initiation) to about nucleotide 100 of the transcript.

Because of the degeneracy of the genetic code, there is substantial freedom in codon choice for any given amino acid sequence. Given this freedom, the number of different DNA nucleotide sequences encoding any given amino acid sequence is exceedingly large, for example, upwards of 2.6×10^5

possibilities for somatostatin consisting of only 14 amino acids. Again, the present invention provides methods and means for selecting certain of these DNA sequences, those which will efficiently prepare functional product. For a given polypeptide product hereof, the present invention provides means to select, from among the large number possible, those DNA sequences that provide transcripts, the conformational structure of which admits of accessiblity for operable and efficient ribosomal translation.

Conformational structure of mRNA transcripts is a consequence of hydrogen bonding between complementary nucleotide sequences that may be separated one from another by a sequence of noncomplementary nucleotides. Such bonding is commonly referred to as secondary structure. So-called tertiary structures may add to the conformation of the overall molecule. These structures are believed to be a result of spatial interactions within one or more portions of the molecule -- so-called stacking interactions. In any event, the conformational structure of a given mRNA molecule can be determined and measured. Furthermore, we have now discovered that certain levels of conformational structure of mRNA transcripts interfere with efficient protein synthesis, thus effectively blocking the initiation and/or continuation of translation (elongation) into polypeptide product. Accordingly, levels at which such conformational structure does not occur, or at least is minimal, can be predicted. Nucleotide choice can be prescribed on the basis of the predictable, permissible levels of conformational structure, and preferred gene sequences determined accordingly.

The measurement of mRNA conformational structure is determined, in accordance herewith, by measuring the energy levels associated with the conformational structure of the mRNA molecule.

In determining such energy levels, the thermodynamic disassociation energy connected with one or a series of homologous base pairings is calculated, for example according to the rules of Tinoco et al., Nature New Biol 246, 40(1973). In the calculation used herein (not that of Tinoco et al, supra), AT base pairing is assigned an associated energy level of about -1.2 Kcal/mole while a CG base pairing is assigned an associated energy level of about -2 Kcal/mole. Adjacent homologous pairings are more than additive, doubtless due to stacking interactions and other associative factors. In any event, it has been determined that in those instances where, according to this calculation regional base pairing interactions result in energy levels stronger than about -12 kcal/mole (that is, values expressed arithmetically in numbers less than about -12 kcal/mole) for a given homologous sequence, such interactions are likely sufficient to hinder or block the translation phase of expression, most probably by interfering with accessibility for necessary ribosomal binding.

A given DNA sequence is screened as follows: A first series of base pairs, e.g., approximately the first six base pairs, are compared for homology with the corresponding reverse last base pairs of the gene. If such homology is found, the associate energy levels are calculated according to the above considerations. The first series of base pairs is next compared with the corresponding last base pairs up to the penultimate base pair of the gene and the associative energy levels of any homology calculated. In succession the first series of base pairs is next compared with the corresponding number of base pairs up to the antepenultimate base pair, and so on until the entire gene sequence is compared, back to front. Next, the series of base pairs beginning one downstream from the first series, e.g. base pairs 2 to 7 of the prior example, is compared with the corresponding number from the end and progressively toward the front of the gene, as described above. This procedure is repeated until each base pair is compared for homology with all other regions of the gene and associated energy levels are determined. Thus, for example in Figure 3 there are provided results of such scanning and calculating for two genes - those encoding natural bovine growth hormone (BGH) and synthetic (i.e., hybrid) BGH. It can be seen that natural BGH contains two regions of homology considered relevant herein (i.e., according to this calculation, energy level greater than about -12 kcal/mole), to wit, six base pairs from base pair 33 to 38 with homologous pairs 96 to 101 and six base pairs from 46 to 51 with 73 to 78. The first is not significant for present purpose, despite the energy level (-15.40 kcal/mole), presumably because the region of homology lies downstream a sufficient distance so as not to be influential on translation efficiency. The second region is significant as evidenced by the poor yields of product as described herein cf. infra. The synthetic BGH gene where such region of homology was eliminated provided good yields of intended protein.

An embodiment of the present invention will now be described by way of example with reference to the accompanying drawings, in which:

Figure 1 depicts the amino acid and nucleotide sequences of the proximal portions of natural BGH, synthetic HGH, and synthetic BGH. The amino acids and nucleotides in natural BGH that are different from those in synthetic HGH are underlined. The nucleotides in the proximal portion of the synthetic BGH gene that differ from those in the natural BGH gene also are underlined. The position of the PVUII restriction site at the end of the proximal portion of these genes is indicated.

In arriving at the synthetic BGH gene encoding the proper amino acid sequence for BGH, the

nucleotide sequences of natural BGH and synthetic HGH were compared. Nucleotide selections were made based upon the synthetic HGH gene for construction of the synthetic BGH gene taking into account also the latitude permitted by the degeneracy of the genetic code, using a minimum of nucleotide changes from the synthetic HGH sequence.

Figure 2 depicts the nucleotide sequences of the sense strands of both natural and synthetic BGH genes along with the transcribed portions of the respective preceding trp-promotor sequences. The first nucleotide of each transcript is indicated as "+1" and the following nucleotides are numbered sequentially. The sequences are lined up to match the translated coding regions of both genes, beginning at the start codon "ATG" of each (overlined). The transcript of the natural BGH gene shows an area of "secondary structure" due to interactions of nucleotides 46 to 51 with nucleotides 73 to 78, respectively (see Figure 3), thus creating the stem-loop structure depicted. This area is not present in the synthetic BGH gene, removed by virtue of nucleotide changes (see Figure 1), which nevertheless retains the correct amino acid sequence.

Figure 3 shows the locations and stabilities of secondary structures in the transcripts of natural and synthetic BGH. (See Figure 2) These locations and stabilities were determined using a nucleotide by nucleotide analysis, as described herein. Each area of significant secondary structure of each proximal portion of gene is listed in the respective table. Thus, for natural BGH versus synthetic BGH, it is noted that the energy levels of "secondary structure" at corresponding portions of the translatable transcripts (namely, nucleotides 46 to 78 comprising a 6 nucleotide long stem in natural BGH versus nucleotides 52 to 84 of synthetic BGH) are markedly different (according to this calculation -15.2 kcal/mole versus greater than -10 kcal/mole), accounting for the observed success of expression of the synthetic BGH gene versus the natural BGH gene, cf. infra. The energy levels indicate the significance of the relative amounts of tolerable "secondary structure", i.e., according to this calculation values arithmetically greater than about -12kcal/mole based upon thermodynamic energy considerations. The significance of location of "secondary structure" can be appreciated by the fact that energy levels calculated for positions 33 to 101 versus 38 to 104 of natural versus synthetic BGH, respectively, did not significantly influence expression levels.

Figure 4 depicts the construction of pBGH 33 used as shown in Figure 5.

Figure 5 depicts the construction of plasmids harboring DNA sequences for hybrid polypeptides: pBHGH 33-1 used as shown in Figure 7, pBHGH being a hybrid of bovine and human growth hormone sequences, and pHBGH a hybrid of human and bovine sequences.

Figure 6 depicts the technique used to assemble the synthetic proximal portion of the BGH gene, pBR 322-01, used in the construction shown in Figure 7.

Figure 7 depicts the construction of the plasmid (pBGH 33-3) harboring the gene for BGH comprising the synthetic proximal portion as shown in Figure 6.

Figure 8 depicts the construction of expression plasmid pBGH 33-4 harboring the hybrid BGH gene.

Figure 9 is the result of a polyacrylamide gel segregation of cell protein. Part A shows no BGH production at any cell density using the culture containing natural BGH gene. Part B shows the expression of synthetic BGH gene (lanes BGH #1 and #2) in the same medium as used for Part A. The levels of expression indicated in Part B, as opposed to Part A, reflect the production of BGH in amounts exceeding about 100 thousand copies per cell.

In its most preferred embodiment, the invention is illustrated by the microbial production of bovine growth hormone (BGH). BGH is endogenous in bovine, e.g., cattle, and is responsible for proper physical maturation of the animal. It is also useful for increasing weight gain, feed conversion efficiency, lean to fat ratio, and milk production. Its sequence of 190 amino acids is known. See Dayhoff, Atlas of Protein Sequence and Structure 1972, National Biomedical Research Foundation, Washington, D.C. The present invention makes possible the preparation of commercial quantities of the compound, enabling now its application on a large scale in the animal husbandry industry. An initial approach toward preparing BGH microbially took advantage of a source of bovine pituitary glands. By extraction and purification, the requisite mRNA for BGH was isolated and from it, corresponding cDNA prepared. Thus, this initial work resulted in a gene corresponding, for all intents and purposes, to the natural DNA sequence of BGH. After removal of DNA coding for the presequence and adding a start codon, the cDNA was ligated to a plasmid vector under proper control of a promotor. This plasmid was used to transform E. coli host which was grown under usual conditions. The efficiency of expression of BGH product was poor, a consequence, it was discovered, of conformational structure of the messenger RNA, which greatly reduced its accessibility for ribosomal translation, cf. Figure 3.

For example, it was found that in "natural" BGH mRNA there are regions of complementary homology. One significant region centers around positions +46 to +51 with a homologous region at positions +73 to +78. Secondary structure considerations, in these two defined regions, are thought to create a hairpin

arrangement just downstream from the translation start codon ATG and the ribosome binding site. This conformational arrangement interferes with or prematurely disrupts ribosomal binding, and hence, inhibits translation.

The recognition of this phenomenon prompted investigations into the nature of the DNA sequence in these regions and the discovery of methods and moans to obviate the problem. In accordance herewith, advantage was taken of a Pvu II endonuclease restriction site at the BGH DNA corresponding to amino acid 24. DNA for the first 24 amino acids of BGH was chemically synthesized, the selection of nucleotides taking into strict account proper coding sequence and resultant mRNA secondary/ tertiary structure considerations. Employing the method defined above, it was found that certain nucleotide base selections would be suitable, on the basis of predicted conformational structure energy levels, to prepare gene sequences properly encoding BGH but devoid of problematic conformational structure. One of these was selected and synthesized. Ligations at the Pvu II terminus of the synthetic piece to the cDNA downstream therefrom produced the desired hybrid gene. Construction of a replicable expression vector containing said heterologous gene as an operable insert successfully resulted in efficient expression of BGH in transformed E. coli host.

The complete nucleotide (and deduced amino acid) sequence of the thus constructed hybrid BGH gene is as follows:

```
     1
met phe pro ala met ser leu ser gly leu phe ala asn ala val
ATG TTC CCA GCT ATG TCT CTA TCT GGT CTA TTC GCT AAC GCT GTT

                        20
leu arg ala gln his leu his gln leu ala ala asp thr phe lys
CTT CGT GCT CAG CAT CTT CAT CAG CTG GCT GCT GAC ACC TTC AAA

                                                40
glu phe glu arg thr tyr ile pro glu gly gln arg tyr ser ile
GAG TTT GAG CGC ACC TAC ATC CCG GAG GGA CAG AGA TAC TCC ATC

gln asn thr gln val ala phe cys phe ser glu thr ile pro ala
CAG AAC ACC CAG GTT GCC TTC TGC TTC TCT GAA ACC ATC CCG GCC

 60
pro thr gly lys asp glu ala gln gln lys ser asp leu glu leu
CCC ACG GGC AAG GAT GAG GCC CAG CAG AAA TCA GAC TTG GAG CTG

                        80
leu arg ile ser leu leu leu ile gln ser trp leu gly pro leu
CTT CGC ATC TCA CTG CTC CTC ATC CAG TCG TGG CTT GGG CCC CTG

                                                100
gln phe leu ser arg val phe thr asn ser leu val phe gly thr
CAG TTC CTC AGC AGA GTC TTC ACC AAC AGC TTG GTG TTT GGC ACC

ser asp arg val tyr glu lys leu lys asp leu glu glu gly ile
TCG GAC CGT GTC TAT GAG AAG CTG AAG GAC CTG GAG GAA GGC ATC

120
leu ala leu met arg glu leu glu asp gly thr pro arg ala gly
CTG GCC CTG ATG CGG GAG CTG GAA GAT GGC ACC CCC CGG GCT GGG

                        140
gln ile leu lys gln thr tyr asp lys phe asp thr asn met arg
CAG ATC CTC AAG CAG ACC TAT GAC AAA TTT GAC ACA AAC ATG CGC

                                                160
ser asp asp ala leu leu lys asn tyr gly leu leu ser cys phe
AGT GAC GAC GCG CTG CTC AAG AAC TAC GGT CTG CTC TCC TGC TTC

arg lys asp leu his lys thr glu thr tyr leu arg val met lys
CGG AAG GAC CTG CAT AAG ACG GAG ACG TAC CTG AGG GTC ATG AAG

180                                             190
cys arg arg phe gly glu ala ser cys ala phe stop
TGC CGC CGC TTC GGG GAG GCC AGC TGC GCA TTC TAG
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Detailed Description

Synthesis of Proximal Portion of BGH Gene

Twelve fragments, U 1-6 (upper strand) and L 1-6 (lower strand), were synthesized Also synthesized, in order to repair the 3' end of the gene, were 2 fragments, BGH Repair (1) (upper strand) and BGH Repair (2) (lower strand).

The 14 fragments were synthesized according to the method of Crea et al., Nucleic Acids Research, 8, 2331 (1980). The syntheses of the fragments were accomplished from the appropriate solid support (cellulose) by sequential addition of the appropriate fully protected diner - or trimer- blocks. The cycles were carried out under the same conditions as described in the synthesis of oligothymidilic acid (see Crea et al., Supra.) The final polymer was treated with base (aq. conc NH_3) and acid (80% aq. HOAC), the polymer pelleted off and the supernatant evaporated to dryness. The residue, as dissolved in 4% aq. NH_3, was washed with ether (3x) and used for the isolation of the fully deprotected fragment. Purification was accomplished by hplc on Rsil NH_2 u-particulate column. Gel electrophoretic analysis showed that each of

the fragments, U,L 1-6 and BGH Repair (1) and (2), had the correct size:

Fragment	Sequence	Size
U 1	5' AAT.TCT.ATG.TTC.C 3'	13-mer
U 2	5' CAG.CTA.TGT.CTC.T 3'	13-mer
U 3	5' ATC.TGG.TCT.ATT.C 3'	13-mer
U 4	5' GCT.AAC.GCT.GTT.C 3'	13-mer
U 5	5' TTC.GTG.CTC.AGC.A 3'	13-mer
U 6	5' TCT.TCA.TCA.GCT.GA 3'	14-mer
L 1	5' ATA.GCT.GGG.AAC.ATA.G 3'	16-mer
L 2	5' ACC.AGA.TAG.AGA.C 3'	13-mer
L 3	5' CGT.TAG.CGA.ATA.G 3'	13-mer
L 4	5' GCA.CGA.AGA.ACA.G 3'	13-mer
L 5	5' ATG.AAG.ATG.CTG.A 3'	13-mer
L 6	5' AGC.TTC.AGC.TG 3'	11-mer
BGH Repair (1)	5' AA.TTC.AGC.TGC.GCA.TTC.TAG.A 3'	21-mer
BGH Repair (2)	5' AG.CTT.CTA.GAA.TGC.GCA.GCT.G 3'	21-mer

Construction of pBGH 33 (Fig. 4)

Fresh frozen bovine pituitaries were macerated and RNA was extracted by the guanidium thiocyanate method. (Harding et al., J. Biol Chem. 252 (20), 7391 (1977) and Ullrich et al., Science 196, 1313 (1977)). The total RNA extract was then passed over an oligo-dT cellulose column to purify poly A containing messenger RNA (mRNA). Using reverse transcriptase and oligo-dT as a primer, single stranded cDNA was made from the mRNA. Second strand synthesis was achieved by use of the Klenow fragment of DNA polymerase I. Following S1 enzyme treatment and acrylamide gel electrophoresis a size cut of the total cDNA (ca. 500-1500 bp) was eluted and cloned into the Pst I site of the amp^R gene of pBR 322 using traditional tailing and annealing conditions.

The pBR 322 plasmids containing cDNA were transformed into E. coli K-12 strain 294 (ATCC No. 31446). Colonies containing recombinant plasmids were selected by their resistance to tetracycline and sensitivity to ampicillin. Approximately 2000 of these clones were screened for BGH by colony hybridization.

The cDNA clones of HGH contain an internal 550 bp HaeIII fragment. The amino acid sequence of this region is very similar to the BGH amino acid sequence. This HGH HaeIII fragment was radioactively labeled and used as a probe to find the corresponding BGH sequence amongst the 2000 clones.

Eight positive clones were identified. One of these, pBGH112, was verified by sequence analysis as BGH. This full-length clone is 940 bp long containing the coding region of the 26 amino acid presequence as well as the 191 amino acid protein sequence.

In order to achieve direct BGH expression, a synthetic "expression primer" was made having the sequence 5'-ATGTTCCCAGCCATG-3'. The nucleotides in the fourth through fifteenth position are identical to the codons of the first 4 amino acids of the mature BGH protein, as determined by sequence data of pBGH 112 . Only the 5' ATG (methionine) is alien to this region of the protein. This was necessary in order to eliminate the presequence region of our BGH clone and to provide the proper initiation codon for protein synthesis. By a series of enzymatic reactions this synthetic primer was elongated on the BGH 112 cDNA insert. The primed product was cleaved with Pst I to give a DNA fragment of 270 bp containing coding information up to amino acid 90. (Figure 4) This "expression" BGH cDNA fragment was ligated into a pBR

322 vector which contained the trp promotor. This vector was derived from pLeIF A trp25 (Goeddel et al., Nature 287, 411 (1980)). The interferon cDNA was removed and the trp25-322 vector purified by gel electrophoresis. The recombinant plasmid (pBGH710) now contained the coding information for amino acids 1-90 of the mature BGH protein, linked directly to the trp promotor. This linkage was verified by DNA sequence analysis. The second half of the coding region and the 3' untranslated region was isolated from pBGH112 by PstI restriction digest and acrylamide gel electrophoresis. This "back-end" fragment of 540 bp was then ligated into pBGH710 at the site of amino acid 90. Recombinant plasmids were checked by restriction analysis and DNA sequencing. The recombinant plasmid, pBGH33, has the trp promotor directly linked via ATG with the complete DNA coding sequence for mature BGH.

Construction of pHGH 207-1

Plasmid pGMI carries the E. coli tryptophan operon containing the deletion LE1413 (G.F. Miozzari, et al., (1978) J. Bacteriology 145-1466)) and hence expresses a fusion protein comprising the first 6 amino acids of the trp leader and approximately the last third of the trp E polypeptide (hereinafter referred to in conjunction as LE'), as well as the trp D polypeptide in its entirety, all under the control of the trp promoter-operator system. The plasmid, 20 µg, was digested with the restriction enzyme PvuII which cleaves the plasmid at five sites. The gene fragments were next combined with EcoRI linkers (consisting of a self complementary oligonucleotide of the sequence: pCATGAATTCATG) providing an EcoRI cleavage site for a later cloning into a plasmid containing an EcoRI site. The 20 µg of DNA fragments obtained from pGMI were treated with 10 units T_4 DNA ligase in the presence of 200 pico moles of the 5'-phosphorylated synthetic oligonucleotide pCATGAATTCATG and in 20µl T_4 DNA ligase buffer (20mM tris, pH 7.6, 0.5 mM ATP, 10 mM $MgCl_2$, 5 mM dithiothreitol) at 4°C overnight. The solution was then heated 10 minutes at 70°C to inactivate ligase. The linkers were cleaved by EcoRI digestion and the fragments, now with EcoRI ends, were separated using polyacrylamide gel electrophoresis (hereinafter "PAGE") and the three largest fragments isolated from the gel by first staining with ethidium bromide, locating the fragments with ultraviolet light, and cutting from the gel the portions of interest. Each gel fragment, with 300 microliters 0.1xTBE, was placed in a dialysis bag and subjected to electrophoresis at 100 V for one hour in 0.1xTBE buffer (TBE buffer contains: 10.8 gm tris base, 5.5 gm boric acid, 0.09 gm Na_2EDTA in 1 liter H_2O). The aqueous solution was collected from the dialysis bag, phenol extracted, chloroform extracted and made 0.2 M sodium chloride, and the DNA recovered in water after ethanol precipitation. (All DNA fragment isolations hereinafter described are performed using PAGE followed by the electroelution method just discussed.) The trp promoter-operator-containing gene with EcoRI sticky ends was identified in the procedure next described, which entails the insertion of fragments into a tetracycline sensitive plasmid which, upon promoter-operator insertion, becomes tetracycline resistant.

Plasmid pBRH1, (R.I. Rodriguez, et al., Nucleic Acids Research 6, 3267-3287 [1979]) expresses ampicillin resistance and contains the gene for tetracycline resistance but, there being no associated promoter, does not express that resistance. The plasmid is accordingly tetracycline sensitive. By introducing a promoter-operator system in the EcoRI site, the plasmid can be made tetracycline resistant.

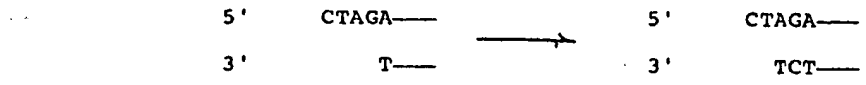
pBRH1 was digested with EcoRI and the enzyme removed by phenol/$CHCl_3$ extraction followed by chloroform extraction and recovered in water after ethanol precipitation. The resulting DNA molecule was, in separate reaction mixtures, combined with each of the three DNA fragments obtained as decribed above and ligated with T_4 DNA ligase as previously described. The DNA present in the reaction mixture was used to transform competent E. coli K-12 strain 294 (K. Backman et al., Proc Nat'l Acad Sci USA 73, 4174-4198 (1976) (ATCC no. 31446) by standard techniques (V. Hershfield et al., Proc Nat'l Acad Sci USA 71, 3455-3459 (1974) and the bacteria plated on LB plates containing 20 µg/ml ampicillin and 5 µg/ml tetracycline. Several tetracycline-resistant colonies were selected, plasmid DNA isolated and the presence of the desired fragment confirmed by restriction enzyme analysis. The resulting plasmid, designed pBRHtrp, expresses β-lactamase, imparting ampicillin resistance, and it contains a DNA fragment including the trp promoter-operator and encoding a first protein comprising a fusion of the first six amino acids of the trp leader and approximately the last third of the trp E polypeptide (this polypeptide is designated LE'), and a second protein corresponding to approximately the first half of the trp D polypeptide (this polypeptide is designated D'), and a third protein coded for by the tetracycline resistance gene.

pBRH trp was digested with EcoRI restriction enzyme and the resulting fragment 1 isolated by PAGE and electroelution. EcoRI-digested plasmid pSom 11 (K. Itakura et al, Science 198, 1056 (1977); G.B. patent publication no. 2 007 676 A) was combined with this fragment 1. The mixture was ligated with T_4 DNA ligase as previously described and the resulting DNA transformed into E. coli K-12 strain 294 as previously described, Transformant bacteria were selected on ampicillin-containing plates. Resulting ampicillin-resistant

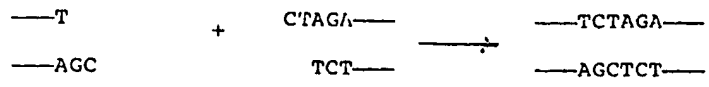
colonies were screened by colony hybridization (M. Gruenstein et al., Proc Nat'l Acad Sci USA 72, 3951-3965 [1975]) using as a probe the trp promoter- operator-containing fragment 1 isolated from pBRHtrp, which had been radioactively labelled with P^{32}. Several colonies shown positive by colony hybridization were selected, plasmid DNA was isolated and the orientation of the inserted fragments determined by restriction analysis employing restriction enzymes BglII and BamHI in double digestion. E. coli 294 containing the plasmid designated pSOM7Δ2, which has the trp promoter-operator fragment in the desired orientation was grown in LB medium containing 10 µg/ml ampicillin. The cells were grown to optical density 1 (at 550 nM), collected by centrifugation and resuspended in M9 media in tenfold dilution. Cells were grown for 2-3 hours, again to optical density 1, them lysed and total cellular protein analyzed by SDS (sodium dodcyl sulfate) area (15 percent) polyacrylamide gel electrophoresis (J.V. Maizel Jr. et al., Metb Viral 5, 180-246 (1971)).

The plasmid pSom7Δ2, 10µg, was cleaved with EcoRI and the DNA fragment 1 containing the tryptophan genetic elements was isolated by PAGE and electroelution. This fragment, 2µg, was digested with the restriction endonuclease Taq I, 2 units, 10 minutes at 37°C such that, on the average, only one of the approximately five Tag I sites in each molecule is cleaved. This partially digested mixture of fragments was separated by PAGE and an approximately 300 base pair fragment 2 that contained one EcoRI end and one Tag I end was isolated by electroelution. The corresponding Tag I mite is located between the transcription start and translation start sites and is 5 nucleotides upstream from the ATG codon of the trp leader peptide. The DNA sequence about this site is shown in Figure 4. By proceeding as described, a fragment could be isolated containing all control elements of the trp operon, i.e., promoter-operator system, transcription initiation signal, and part of the trp leader ribosome binding site.

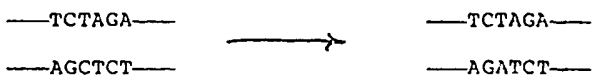
The Tag I residue at the 3' end of the resulting fragment adjacent the translation start signal for the trp leader sequence was next converted into an XbaI site. This was done by ligating the Fragment 2 obtained above to a plasmid containing a unique (i.e., only one) EcoRI site and a unique XbaI site. For this purpose, one may employ essentially any plasmid containing, in order, a replicon, a selectable marker such as antibiotic resistance, and EcoRI, XbaI and BamHI sites. Thus, for example, an XbaI site can be introduced between the EcoRI and BamHI sites of pBR322 (F. Bolivar et al., Gene 2, 95-119 [1977]) by, e.g., cleaving at the plasmid's unique Hind III site with Hind III followed by single strand-specific nuclease digestion of the resulting sticky ends, and blunt end ligation of a self annealing double-stranded synthetic nucleotide containing the recognition site such as CCTCTAGAGG. Alternatively, naturally derived DNA fragments may be employed, as was done in the present case, that contain a single XbaI site between EcoRI and BamHI cleavage residues. Thus, an EcoRI and BamHI digestion product of the viral genome of hepatitis B was obtained by conventional means and cloned into the EcoRI and BamHI sites of plasmid pGH6 (D.V. Goeddel et al., Nature 281, 544 [1979])) to form the plasmid pHS32. Plasmid pHS32 was cleaved with XbaI, phenol extracted, chloroform extracted and ethanol precipitated. It was then treated with 1 µl E. coli polymerase I, Klenow fragment (Boehringer-Mannheim) in 30 µl polymerase buffer (50 mM potassium phosphate pH 7.4, 7mM $MgCl_2$, 1 mM β-mercaptoethanol) containing 0.1mM dTTP and 0.1mM dCTP for 30 minutes at 0°C then 2 hr. at 37°C. This treatment causes 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site to be filled in:



Two nucleotides, dC and dT, were incorporated giving an end with two 5' protruding nucleotides. This linear residue of plasmid pHS32 (after phenol and chloroform extraction and recovery in water after ethanol precipitation) was cleaved with EcoRI. The large plasmid Fragment was separated from the smaller EcoRI-XbaI fragment by PAGE and isolated after electroelution. This DNA fragment from pHS32 (0.2 µg), was ligated, under conditions similar to those described above, to the EcoRI-Taq I fragment of the tryptophan operon (0.01 µg). In this process the Taq I protruding end is ligated to the XbaI remaining protruding end even though it is not completely Watson-Crick base-paired:



A portion of this ligation reaction mixture was transformed into E. coli 294 cells as in part I. above, heat treated and plated on LB plates containing ampicillin. Twenty-four colonies were selected, grown in 3 ml LB media, and plasmid isolated. Six of these were found to have the XbaI site regenerated via E. coli catalyzed DNA repair and replication:



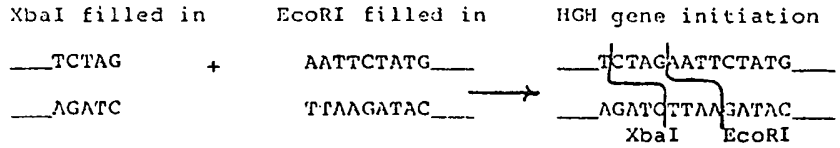
These plasmids were also found to cleave both with EcoRI and HpaI and to give the expected restriction fragments. One plasmid 14, designated pTrp 14, was used for expression of heterologous polypeptides, as next discussed.

The plasmid pHGH 107 (D.V. Goeddel et al, Nature, 281, 544, 1979) contains a gene for human growth hormone made up of 23 amino acid codons produced from synthetic DNA fragments and 163 amino acid codons obtained from complementary DNA produced via reverse transcription of human growth hormone messenger RNA. This gene, 3, though it lacks the codons of the "pre" sequence of human growth hormone, does contain an ATG translation initiation codon. The gene was isolated from 10 µg pHGH 107 after treatment with EcoRI followed by E. coli polymerase I Klenow fragment and dTTP and dATP as described above. Following phenol and chloroform extraction and ethanol precipitation the plasmid was treated with BamHI.

The human growth hormone ("HGH") gene-containing fragment 3 was isolated by PAGE followed by electroelution. The resulting DNA fragment also contains the first 350 nucleotides of the tetracycline resistance structural gene, but lacks the tetracyline promoter-operator system so that, whom subsequently cloned into an expression plasmid, plasmids containing the insert can be located by the restoration of tetracycline resistance. Because the EcoRI end of the fragment 3 has been filled in by the Klenow polymerase I procedure, the fragment has one blunt and one sticky end, ensuring proper orientation when later inserted into an expression plasmid.

The expression plasmid pTrp14 was next prepared to receive the HGH gene-containing fragment prepared above. Thus, pTrp14 was XbaI digested and the resulting sticky ends filled in with the Klenow polymerase I procedure employing dATP, dTTP, dGTP and dCTP. After phenol and chloroform extraction and ethanol precipitation the resulting DNA was treated with BamHI and the resulting large plasmid fragment isolated by PAGE and electroelution. The pTrp14-derived fragment had one blunt and one sticky end, permitting recombination in proper orientation with the HGH gene containing fragment 3 previously described.

The HGH gene fragment 3 and the pTrp14 Xba-BamHI fragment were combined and ligated together under conditions similar to those described above. The filled in XbaI and EcoRI ends ligated together by blunt end ligation to recreate both the XbaI and the EcoRI site:



This construction also recreates the tetracycline resistance gene. Since the plasmid pHGH 107 expresses tetracycline resistance from a promoter lying upstream from the HGH gene (the lac promoter), this construction, designated pHGH 207, permits expression of the gene for tetracycline resistance under the control of the tryptophan promoter-operator. Thus the ligation mixture was transformed into E. coli 294 and colonies selected on LB plates containing 5 µg/ml tetracycline.

Construction of pBGH33-1 (Figure 5)

The structure of pHGH207-1 which has the entire human growth hormone gene sequence is shown. The front part of this gene is synthetic as is described by Goeddel et al., Nature 281, 544 (1979). In the following a plasmid was constructed containing the BGH gene in the same orientation and in the sane position with respect to the trp-promotor as is the HGH gene in pHGH 207-1.

Twenty µl (i.e. 10µg) of the plasmid DNA was digested wth Bam HI and PvuII as follows: To the twenty µl of DNA we added 5 µl 10X restriction enzyme buffer (500mM NaCl, 100 mM Tris HCl pH 7.4, 100 mM $MgSo_4$ and 10 mM DTT), 20 µl H_2O and 10 units BamHI restriction enzyme and 2 µl PvuII restriction enzyme.

Subsequently, this reaction mixture was incubated at 37 °C for 90 minutes. The mixture was loaded on a 6 percent acrylamide gel and electrophoresis was carried out for 2 hours at 50 mA. The DNA in the gel was stained with Ethidium bromide and visualized with UV-light. The band corresponding to the 365 bp (with reference to a HaeIII digest of pBR322) fragment was excised and inserted in a dialysis bag and the DNA was electroeluted using a current of 100 mA. The liquid was removed from the bag and its salt concentration adjusted to 0.3M NaCl. Two volumes of ethanol were added and the DNA precipitated at -70 °C. The DNA was spun down in an Eppendorf centrifuge, washed with 70 percent ethanol and dried and resuspended in 10 µl TAE (10 mM Tris HCl pH7.4, 0.1 mM EDTA). Similarly, the large XbaI Bam HI fragment of pHGH 207-1 and the XbaI, partial PvuII 570 bp fragment of pBGH33 were isolated.

Two µl of each of the thus isolated DNA fragments were mixed. 1 µl 10mM ATP and 1 µl 10x ligase buffer (200 mM Tris HCl pH7.5, 100mM $MgCl_2$, 20 mM DTT) and 1 µl T_4 DNA ligase and 2 µl H_2O were added. Ligation was done over night at 4 °C. This mixture was used to transform competent E. coli K-12 294 cells as follows: 10 ml L-broth was inoculated with E. coli K-12 294 and incubated at 37 °C in a shaker bath at 37 °C. At OD_{550} of 0.8 the cells were harvested by spinning in a Sorvall centrifuge for 5 min. at 6000 rpm. The cell pellet was washed/resuspended in 0.15 M NaCl, and again spun. The cells were resuspended in 75 mM $CaCl_2$, 5 mM $MgCl_2$ and 10 mM Tris HCl pH7.8 and incubated on ice for at least 20 min. The cells were spun down for 5 min at 2500 rpm and resuspended in the same buffer. To 250 µl of this cell suspension each of the ligation mixtures was added and incubated for 60 min on ice. The cells were heat shocked for 90 seconds at 42 °C, chilled and 2 ml L-broth was added. The cells were allowed to recover by incubation at 37 °C for 1 hour. 100 µl of this cell suspension was plated on appropriate plates which were subsequently incubated over night at 37 °C. The plasmid structure in several of the colonies thus obtained is shown in Figure 5 (pBGH 33-1).

All further constructions were done using the same procedures, as described above, mutatis mutandis.

Construction of the hybrid growth hormone genes HBGH and BHGH (Figure 5)

The two PvuII sites in the HGH and BGH genes are at identical positions. Exchange of PvuII fragments is possible without changing the reading frame of the messenger RNA of these genes. The large difference in expression of both genes is due to differences in initiation of protein synthesis at the beginning of the messages. Therefore, the front part of both genes were exchanged thus constructing hybrid genes that upon transcription would give hybrid messenger RNAs. The two plasmids, pBHGH and pHBGH, were constructed as follows:

From pHGH207-1 there were isolated the large BamHI-XbaI fragment and the 857 bp BamHI (partial) PvuII fragment containing the HGH gene without its front part. From pBGH33-1 there was isolated the 75 bp XbaI-PvuII fragment that contains the front part of the BGH gene. After ligation and transformation pBHGH was obtained. pHBGH was constructed in a similar way to pBHGH; in this case the back part was derived from pBGH33-1 whereas the front part, the 75 bp XbaI-PvuII fragment, was derived from pHGH207-1.

Design and cloning of the synthetic front part of the BGH gene (Figure 6)

The DNA sequence up to the PvuII site of the BGH and HGH genes codes for 22 amino acids. Since the front part of the HGH gene had excellent protein synthesis initiation properties, the sequence of the front part of BGH was designed such that the number of nucleotide changes in the BGH gene would be minimal with respect to the HGH gene. Only 14 base pair changes from the natural BGH sequence were made in order to code for the proper BGH amino acid sequence and reduce conformational structure in the prospective mRNA. The DNA sequence is shown in Figure 6. The sequence ends with EcoRI and HindIII sticky ends to make cloning in a vector easy. Close to the HindIII site is a PvuII site for the proper junction with the remaining part of the BGH gene.

The fragments U1 to U6 and L1 to L6 were synthesized chemically according to the procedures described above. All the fragments except U1 and L6 were mixed and kinased. After addition of U1 and L6 the mixed fragments were ligated, purified on a 6 percent polyacrylamide gel and the 75 bp band extracted and isolated according to standard procedures. This fragment was inserted into pBR322 that had been cut with EcoRI and HindIII. Thus plasmid pBR322-01 was obtained.

Replacement of the natural front part of the BGH gene by the synthetic front part. (Figure 7)

From pBR322-01 the cloned synthetic front of the BGH gene was excised with EcoRI and PvuII, and the resulting 70 bp fragment was isolated. From pBGH33-1 the large EcoRI-BamHI fragment and the 875 bp BamHI (partial) PvuII fragment was isolated. The three fragments were isolated and ligated and used to transform E. coli K-12 294 as described before. Thus, pBGH33-2 was obtained. This plasmid contains the entire BGH gene but does not have a promotor. Therefore, pBGH33-2 was cut with EcoRI and the trp-promotor containing 310 bp EcoRI fragment derived from pHGH207-1 was inserted by ligation. After transformation tetracycline resistant colonies were analyzed. Therefore, these colonies had the inserted trp-promotor oriented towards the HGH- and tet-gene as shown in the figure.

Repair of the 3'-end of the BGH gene. (Figure 8)

The sequences beyond the second PvuII site of the BGH gene are derived from the HGH gene. One of the amino acids at the end is different from that in the natural BGH gene. This 3'-end was repaired as follows. A synthetic DNA fragment as shown was synthesized. It is flanked by an EcoRI and a HindIII end to facilitate cloning and contains a PvuII site and 3 amino acid codons and a stop codon in the reading frame of the BGH gene itself. This fragment was inserted into EcoRI-HindIII opened pBR322. Thus pBR322-02 was obtained. Subsequently this plasmid was cut with PvuII and BamHI and the 360 bp fragment was isolated. From pBGH33-3, which has the entire BGH gene with the synthetic front part, the large BamHI and XbaI fragment and the 570 bp XbaI (partial) PvuII fragment was isolated. These three fragments were ligated and used to transform cells. Thus, pBGH33-4 was obtained. In this plasmid a unique HindIII site is present between the stop codon of the BGH gene and the start codon of the tet-mRNA. Both genes are transcribed under direction of the trp promotor.

A typical growth medium used to derepress and produce high levels of BGH per liter (Figure 9) contains: 5.0 g $(NH_4)_2SO_4$, 6.0 g K_2HPO_4, 3.0 g $NaH_2PO_4.2H_2O$, 1.0 g sodium citrate, 2.5 g glucose, 5 mg tetracycline, 70 mg thiamine HCl, and 60 g $MgSO_4.7H_2O$.

While the present invention has been described, in its preferred embodiments, with reference to the use of E. coli transformants, it will be appreciated that other microorganisms can be employed mutatis mutandis. Examples of such are other E. coli organisms, e.g. E. coli B., E. coli W3110 ATCC No. 31622 (F^-, λ-, gal^-, prototroph), E. coli x 1776, ATCC No. 31537, E. coli D1210, E. coli RV308, ATCC No. 31608, etc., Bacillus subtilis strains, Pseudomonas strains, etc. and various yeasts, e.g., Saccharomyces cerevisiae many of which are deposited and (potentially) available from recognized depository institutions e.g., ATCC. Following the practice of this invention and the final expression of intended polypeptide product, extraction and purification techniques may be those customarily employed in this art, known per se.

Claims

1. A method of improving the translational efficiency of a microbial messenger RNA encoding a heterologous functional polypeptide or a bioactive portion thereof, the method comprising:
   (a) determining the thermodynamic energies of regional base pairing interactions in the messenger RNA corresponding to a DNA sequence within the region extending from the transcription initiation site to nucleotide +100 of the DNA encoding the N-terminal portion of said polypeptide;
   and in accordance with said determination,
   (b) providing a synthetic DNA sequence characterized in that the nucleotides thereof are selected so as to provide, on transcription, corresponding messenger RNA encoding a sequence of amino acids comprising that encoded by the DNA sequence of step a) and which, by virtue of differences from the sequence of the messenger RNA referred to in step a) demonstrates reduced regional base pairing interaction leading to increased efficiency of ribosomal translation; and
   (c) ligating the DNA of step b) in proper reading frame relation with DNA encoding the C-terminal portion of said polypeptide, so as to provide DNA encoding an amino acid sequence comprising the natural sequence of said polypeptide.

2. The method according to claim 1 wherein the messenger RNA of step b), within the region from nucleotide +1 to +100, is free of secondary structure having a thermodynamic energy arithmetically less than or equal to the thermodynamic energy structure formed by homologous base pairing between nucleotides 46 to 51 and nucleotides 73 to 78 of the mRNA of natural BGH as depicted in Fig. 2 hereof.

3. The method according to claim 1 or claim 2 wherein the first nucleotide of said DNA sequence of step b) corresponds to nucleotide +1 of the corresponding messenger RNA.

4. The method according to claim 1 or claim 2 wherein the first nucleotide of said DNA sequence of step b) corresponds to a nucleotide of the translational start signal.

5. The method according to claim 1 or claim 2 wherein said DNA sequence of step b) extends from about the translational start signal to about 75 or more nucleotides downstream thereof.

6. The method of any one of the preceding claims wherein said heterologous functional polypeptide is bovine growth hormone.

7. The method of claim 6 wherein the bovine growth hormone lacks the BGH presequence.

8. The method according to any of the preceding claims wherein the DNA sequence of step b) is as depicted in Figure 1 as "BGH synthetic".

9. The method according to any one of the preceding claims wherein the resulting DNA sequence is inserted together with appropriately positioned translational start and stop signals into a microbial expression vector and is therein brought under the control of a microbially operable promoter, to provide the corresponding microbial expression vehicle.

10. The method according to claim 9 wherein a microorganism is transformed with said microbial expression vehicle to provide the corresponding transformed microorganism.

11. The method according to claim 10 wherein the resulting transformed microorganism is grown under suitable fermentation conditions and caused to produce said polypeptide, said polypeptide being subsequently recovered from the fermentation medium.

12. A method of producing bovine growth hormone which comprises culturing a microorganism to express DNA contained therein encoding a mature bovine growth hormone, wherein the coding sequence within the region up to nucleotide +100 of the mRNA has been altered from that of the natural mRNA sequence of bovine growth hormone, but without altering the natural amino acid sequence, so that the resulting mRNA has conformational structure which, compared with the use of the corresponding natural bovine growth hormone coding sequence, interferes less with expression of the hormone in said microorganism.

13. A method of producing bovine growth hormone which comprises culturing a microorganism to express DNA contained therein encoding a mature bovine growth hormone, wherein the coding sequence within the about 25 N-terminal amino acids is provided by synthetic DNA whose nucleotide sequence has been altered from that of the natural nucleotide sequence of bovine growth hormone, but without altering the natural amino acid sequence, so that the resulting mRNA has conformational structure which, compared with the use of the corresponding natural bovine growth hormone coding sequence, interferes less with expression of the hormone in said microorganism.

14. The method of any one of claims 10 to 13 wherein the nucleotides that encode alanine within the proline-alanine-methionine sequence near the N-terminus of the hormone are GCT, rather than the GCC of the naturally occuring bovine growth hormone DNA.

15. The method of any one of claims 10 to 14 wherein said microorganism is an E.coli strain.

16. The method of any one of claims 10 to 15, wherein the bovine growth hormone is recovered and purified.

Patentansprüche

1. Verfahren zum Verbesseren der Translationswirksamkeit einer mikrobiellen Messenger-RNA, die für ein heterologes funktionales Polypeptid oder einen bioaktiven Abschnitt davon kodiert, wobei das Verfahren

umfaßt:

(a) das Bestimmen der thermodynamischen Energien regionaler Basenpaarungswechselwirkungen in der Messenger-RNA, die einer DNA-Sequenz innerhalb des Bereiches entspricht, der sich von der Transkriptionseinleitungsstelle zum Nukleotid +100 der DNA erstreckt, die für den N-terminalen Abschnitt des genannten Polypeptids kodiert;

und gemäß der genannten Bestimmung,

(b) das Schaffen einer synthetischen DNA-Sequenz, die dadurch gekennzeichnet ist, daß die Nukleotide davon so ausgewählt sind, daß bei der Transkription entsprechende Messenger-RNA geschaffen wird, die für eine Sequenz von Aminosäuren kodiert, die jene umfassen, für welche die DNA-Sequenz von Schritt (a) kodiert, und die, dank der Unterschiede zur Sequenz der Messenger-RNA, auf die in Schritt (a) bezuggenommen wird, verringerte regionale Basenpaarungswechselwirkung zeigt, die zu erhöhter Wirksamkeit der ribosomalen Translation führt; und

(c) das Ligieren der DNA von Schritt (b) in richtige Leserasterbeziehung mit DNA, die für den C-terminalen Abschnitt des genannten Polypeptids kodiert, um DNA zu schaffen, die für eine Aminosäuresequenz kodiert, welche die natürliche Sequenz des genannten Polypeptids umfaßt.

2. Verfahren nach Anspruch 1, worin die Messenger-RNA von Schritt (b) innerhalb des Bereiches von Nukleotid +1 bis +100 frei von sekundärer Struktur ist, die eine thermodynamische Energie aufweist, die arithmetisch geringer oder gleich groß wie die thermodynamische Energiestruktur ist, die durch homologe Basenpaarung zwischen den Nukleotiden 46 bis 51 und den Nukleotiden 73 bis 78 der mRNA von natürlichem BGH, wie in Figur 2 hiervon abgebildet, gebildet wird.

3. Verfahren nach Anspruch 1 oder 2, worin das erste Nukleotid der genannten DNA-Sequenz von Schritt (b) dem Nukleotid +1 der entsprechenden Messenger-RNA entspricht.

4. Verfahren nach Anspruch 1 oder 2, worin das erste Nukleotid der genannten DNA-Sequenz von Schritt (b) einem Nukleotid des translationalen Startsignals entspricht.

5. Verfahren nach Anspruch 1 oder 2, worin die genannte DNA-Sequenz von Schritt (b) sich von etwa dem translationalen Startsignal bis etwa 75 oder mehr Nukleotide stromabwärts davon erstreckt.

6. Verfahren nach einem der vorhergehenden Ansprüche, worin das genannte heterologe funktionale Polypeptid Rinderwachstumshormon ist.

7. Verfahren nach Anspruch 6, worin dem Rinderwachstumshormon die BGH-Präsequenz fehlt.

8. Verfahren nach einem der vorhergehenden Ansprüche, worin die DNA-Sequenz von Schritt (b) wie in Figur 1 als "BGH-synthetisch" dargestellt ist.

9. Verfahren nach einem der vorhergehenden Ansprüche, worin die resultierende DNA-Sequenz gemeinsam mit auf geeignete Weise angeordneten translationalen Start- und Stopsignalen in einen mikrobiellen Expressionsvektor eingefügt wird und darin unter die Kontrolle eines mikrobiell operablen Promotors gebracht wird, um ein entsprechendes mikrobielles Expressionsvehikel zu schaffen.

10. Verfahren nach Anspruch 9, worin ein Mirkoorganismus mit dem genannten mikrobiellen Expressionsvehikel transformiert wird, um den entsprechenden transformierten Mikroorganismus zu schaffen.

11. Verfahren nach Anspruch 10, worin der resultierende transformierte Mikroorganismus unter geeigneten Fermentationsbedingungen gezüchtet wird und dazu gebracht wird, das genannte Polypeptid zu erzeugen, wobei das genannte Polypeptid in der Folge aus dem Fermentationsmedium rückgewonnen wird.

12. Verfahren zur Herstellung von Rinderwachstumshormon, welches das Kultivieren eines Mikroorganismus umfaßt, um darin enthaltene DNA zu exprimieren, die für ein reifes Rinderwachstumshormon kodiert, worin die Kodierungssequenz innerhalb des Bereiches bis zu Nukleotid +100 der mRNA von jener der natürlichen mRNA-Sequenz von Rinderwachstumshormon geändert worden ist, ohne aber die natürliche Aminosäuresequenz zu ändern, sodaß die resultierende mRNA eine Konformationsstruktur

aufweist, die, verglichen mit der Verwendung der entsprechenden natürlichen Rinderwachstumshormonkodierungssequenz, die Expression des Hormons im genannten Mikroorganismus weniger stört.

13. Verfahren zur Herstellung von Rinderwachstumshormon, welches das Kultivieren eines Mikroorganismus umfaßt, um darin enthaltene DNA zu exprimieren, die für ein reifes Rinderwachstumshormon kodiert, worin die Kodierungssequenz innerhalb der etwa 25 N-terminalen Aminosäuren durch synthetische DNA geschaffen wird, deren Nukleotidsequenz von jener der natürlichen Nukleotidsequenz von Rinderwachstumshormon geändert worden ist, ohne aber die natürliche Aminosäuresequenz zu ändern, sodaß die resultierende mRNA eine Konformationsstruktur aufweist, die, im Vergleich zur Verwendung der entsprechenden natürlichen Rinderwachstumshormonkodierungssequenz, die Expression des Hormons im genannten Mikroorganismus weniger stört.

14. Verfahren nach einem der Ansprüche 10 bis 13, worin die Nukleotide, die für Alanin innerhalb der Prolin-Alanin-Methionin-Sequenz nahe des N-Terminus des Hormons kodieren, GCT sind, und nicht die GCC der natürlich auftretenden Rinderwachstumshormon-DNA.

15. Verfahren nach einem der Ansprüche 10 bis 14, worin der genannte Mikroorganismus ein E.coli-Stamm ist.

16. Verfahren nach einem der Ansprüche 10 bis 15, worin das Rinderwachstumshormon gewonnen und gereinigt wird.

Revendications

1. Procédé pour améliorer l'efficacité de traduction d'un ARN messager microbien codant pour un polypeptide fonctionnel hétérologue ou une portion biologiquement active de ce polypeptide, procédé consistant :
   (a) à déterminer les énergies thermodynamiques des interactions régionales d'appariement de bases dans l'ARN messager corrrespondant à une séquence d'ADN dans la région s'étendant du site d'initiation de transcription au nucléotide +100 de l'ADN codant pour la portion N-terminale dudit polypeptide ;
   et, en fonction de ladite détermination,
   (b) à produire une séquence d'ADN synthétique caractérisée en ce que ses nucléotides sont choisis de manière à produire, par transcription, un ARN messager correspondant codant pour une séquence d'aminoacides comprenant celle codée par la séquence d'ADN de l'étape a) et qui, en raison de différences avec la séquence de l'ARN messager mentionné dans l'étape a), présente une interaction régionale réduite d'appariement de bases conduisant à une efficacité accrue de traduction ribosomale ; et
   (c) à réunir par ligation l'ADN de l'étape b), en rapport dans un cadre de lecture convenable, avec l'ADN codant pour la portion C-terminale dudit polypeptide, de manière à produire un ADN codant pour une séquence d'aminoacides comprenant la séquence naturelle dudit polypeptide.

2. Procédé suivant la revendication 1, dans lequel l'ARN messager de l'étape b), dans la région allant du nucléotide +1 au nucléotide +100, est dépourvu de structure secondaire ayant une énergie thermodynamique arithmétiquement inférieure ou égale à l'énergie thermodynamique de la structure formée par appariement de bases homologues entre les nucléotides 46 à 51 et les nucléotides 73 à 78 de l'ARNm de la BGH naturelle, de la manière représentée sur la figure 2 de la présente invention.

3. Procédé suivant la revendication 1 ou la revendication 2, dans lequel le premier nucléotide de la séquence d'ADN de l'étape b) correspond au nucléotide +1 de l'ARN messager correspondant.

4. Procédé suivant la revendication 1 ou la revendication 2, dans lequel le premier nucléotide de la séquence d'ADN de l'étape b) correspond à un nucléotide du signal d'initiation de traduction.

5. Procédé suivant la revendication 1 ou la revendication 2, dans lequel la séquence d'ADN de l'étape b) s'étend approximativement du signal d'initiation de traduction à approximativement 75 ou plus de 75 nucléotides en aval de ce signal.

6. Procédé suivant l'une quelconque des revendications précédentes, dans lequel le polypeptide fonctionnel hétérologue est l'hormone de croissance bovine.

7. Procédé suivant la revendication 6, dans lequel l'hormone de croissance bovine est dépourvue de la préséquence de BGH.

8. Procédé suivant l'une quelconque des revendications précédentes, dans lequel la séquence d'ADN de l'étape b) est conforme à celle représentée sur la figure 1 sous le nom de "BGH synthétique".

9. Procédé suivant l'une quelconque des revendications précédentes, dans lequel la séquence d'ADN résultante est insérée conjointement avec un signal d'initiation et un signal de terminaison de traduction positionnés de manière appropriée dans un vecteur d'expression microbienne et est mise dans ce vecteur sous le contrôle d'un promoteur fonctionnel dans un micro-organisme, pour produire le vecteur d'expression microbienne correspondant.

10. Procédé suivant la revendication 9, dans lequel un micro-organisme est transformé avec le vecteur d'expression microbienne pour produire le micro-organisme transformé correspondant.

11. Procédé suivant la revendication 10, dans lequel le micro-orqanisme transformé résultant est cultivé dans des conditions convenables de fermentation et amené à produire le polypeptide, ledit polypeptide étant ensuite séparé du milieu de fermentation.

12. Procédé de production d'hormone de croissance bovine, qui consiste à cultiver un micro-organisme pour l'expression d'un ADN présent dans ce micro-organisme codant pour une hormone de croissance bovine mature, dans lequel la séquence codante dans la région allant jusqu'au nucléotide +100 de l'ARNm a été modifiée par rapport à celle de la séquence d'ARNm naturelle de l'hormone de croissance bovine, mais sans modification de la séquence d'aminoacides naturelle, de telle sorte que l'ARNm résultant possède une structure conformationnelle qui, comparativement à l'utilisation de la séquence codant pour l'hormone de croissance bovine naturelle correspondante, interfère moins avec l'expression de l'hormone dans ledit micro-organisme.

13. Procédé de production d'hormone de croissance bovine, qui consiste à cultiver un micro-organisme pour l'expression de l'ADN présent dans ce micro-organisme codant pour une hormone de croissance bovine mature, dans lequel la séquence codante dans la région correspondant approximativement au 25 aminoacides N-terminaux est produite par un ADN synthétique dont la séquence de nucléotides a été modifiée par rapport à la séquence de nucléotides naturelle de l'hormone de croissance bovine, mais sans modifier la séquence d'aminoacides naturelle, de telle sorte que l'ARNm résultant possède une structure conformationnelle qui, comparativement à l'utilisation de la séquence codant pour l'hormone de croissance bovine naturelle correspondante, interfère moins avec l'expression de l'hormone dans ledit micro-organisme.

14. Procédé suivant l'une quelconque des revendications 10 à 13, dans lequel les nucléotides qui codent pour l'alanine dans la séquence proline-alanine-méthionine à proximité de l'extrémité N-terminale de l'hormone sont les nucléotides GCT, au lieu des nucléotides GCC de l'ADN d'hormone de croissance bovine naturelle.

15. Procédé suivant l'une quelconque des revendications 10 à 14, dans lequel le micro-organisme est une souche de E. coli.

16. Procédé suivant l'une quelconque des revendications 10 à 15, dans lequel l'hormone de croissance bovine est séparée et purifiée.

BGH natural

amino acids: Met Phe Pro Ala Met Ser Leu Ser Gly Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His Gln

bases: ATG TTC CCA GCC ATG TCC TTG TCC GGC CTG TTT GCC AAC GCT GTG CTC CGG GCT CAG CAC CTG CAT CAG
PvuII

HGH synthetic

amino acids: Met Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln

bases: ATG TTC CCA ACT ATA CCA CTA TCT CGT CTA TTC GAT AAC GCT ATG CTT CGT GCT CAT CGT CTT CAT CAG
PvuII

BGH synthetic

amino acids: Met Phe Pro Ala Met Ser Leu Ser Gly Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His Gln

bases: ATG TTC CCA GCI ATG TCI CTA TCI GGI CTA TTC GCT AAC GCT GTT CTT CGT GCT CAG CAT CTT CAT CAG
PvuII

FIG. 1.

EP 0 075 444 B1

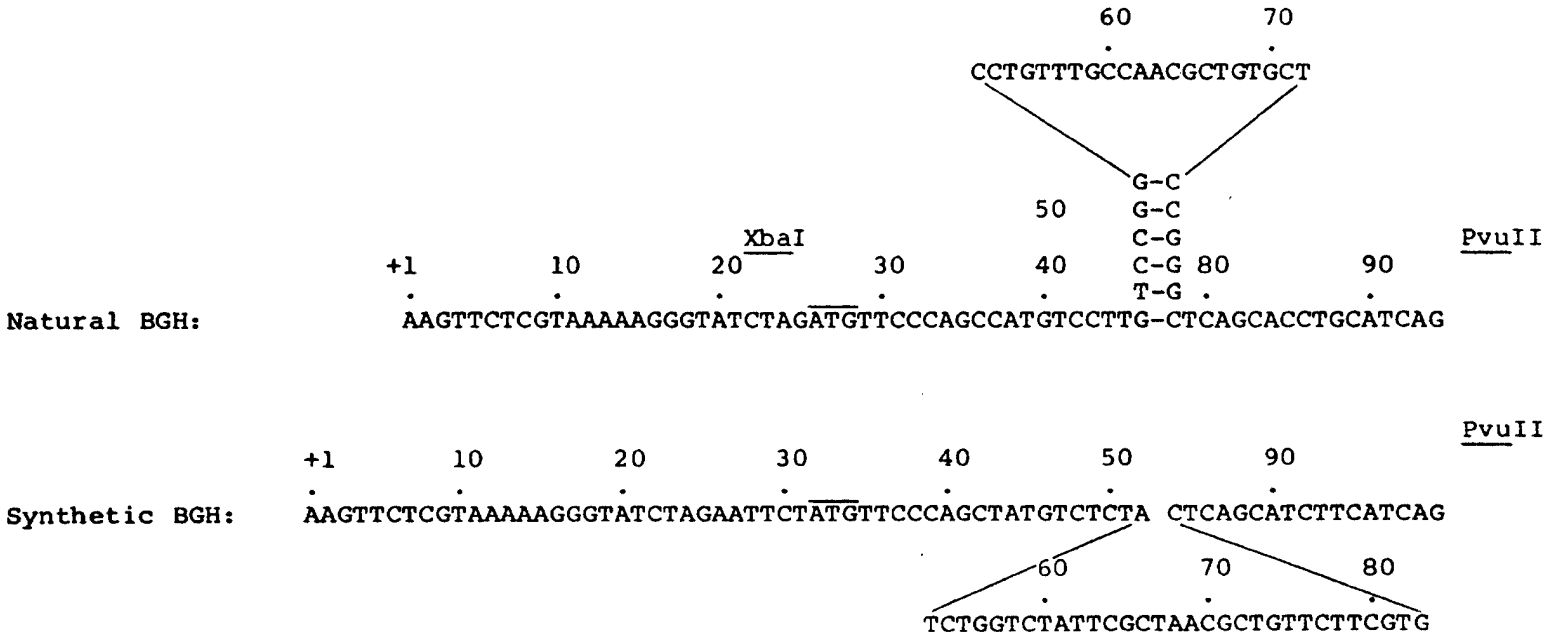


FIG. 2.

EP 0 075 444 B1

Natural BGH				Synthetic BGH			
5'	3'	length	kcal/mol	5'	3'	length	kcal/mol
14	45	8	-11.80	14	79	7	-5.50
16	31	6	4.00	16	37	6	-4.00
33	101	6	-15.40	38	104	6	-15.40
46	78	6	-15.20	52	84	-	>-10

FIG. 3.

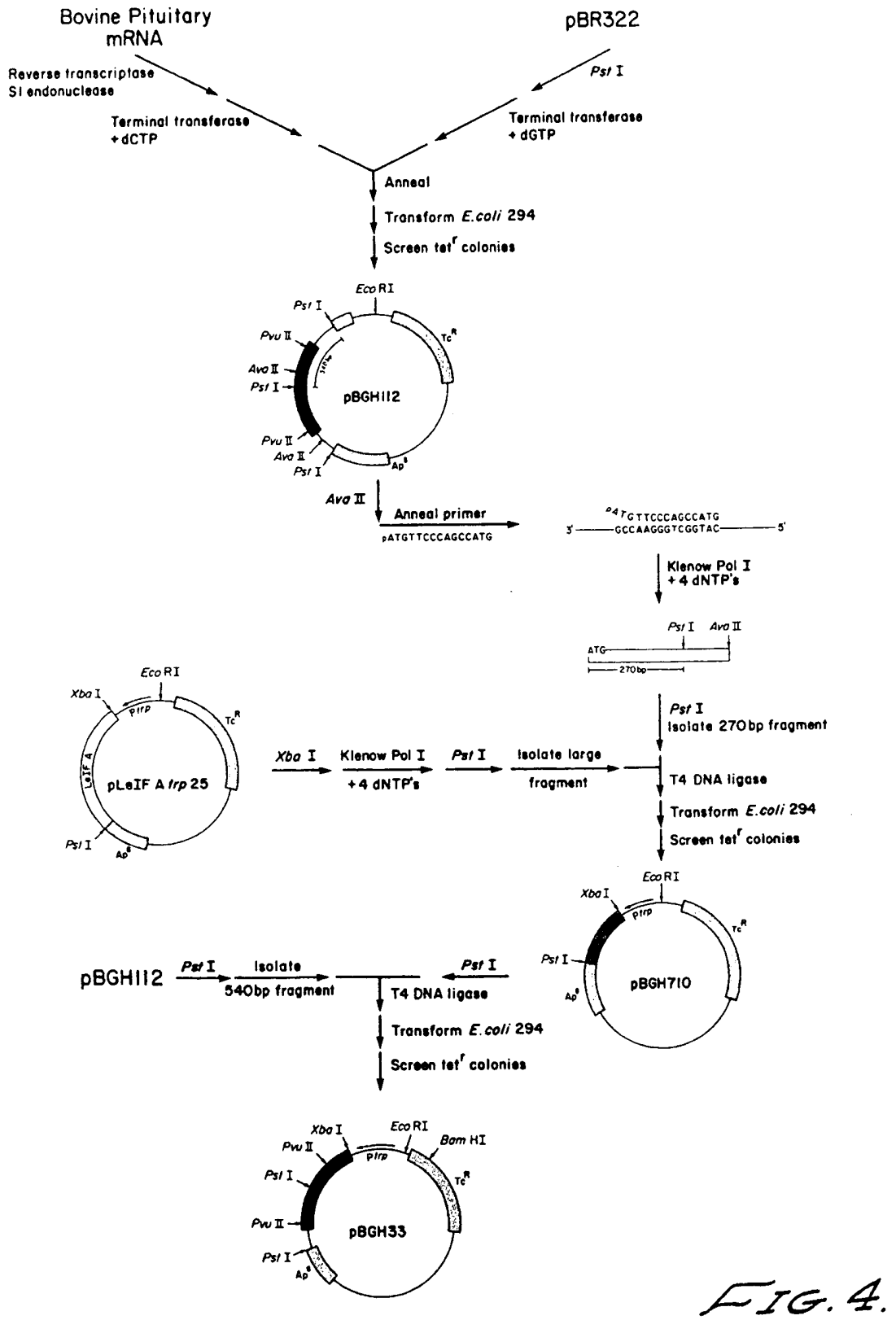

Bovine Pituitary mRNA
pBR322
Reverse transcriptase
S1 endonuclease
Terminal transferase
+dCTP
Pst I
Terminal transferase
+dGTP
Anneal
Transform E.coli 294
Screen tet^r colonies
pBGH112
Ava II
Anneal primer
pATGTTCCCAGCCATG
Klenow Pol I
+4 dNTP's
270bp
Pst I
Isolate 270bp fragment
pLeIF A trp 25
Xba I
Klenow Pol I
+4 dNTP's
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
pBGH710
pBGH112
Isolate
540bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
pBGH33
Eco RI
Bam HI
Pvu II
Ava II
Xba I
trp
Tc^R
Ap^R

FIG. 4.

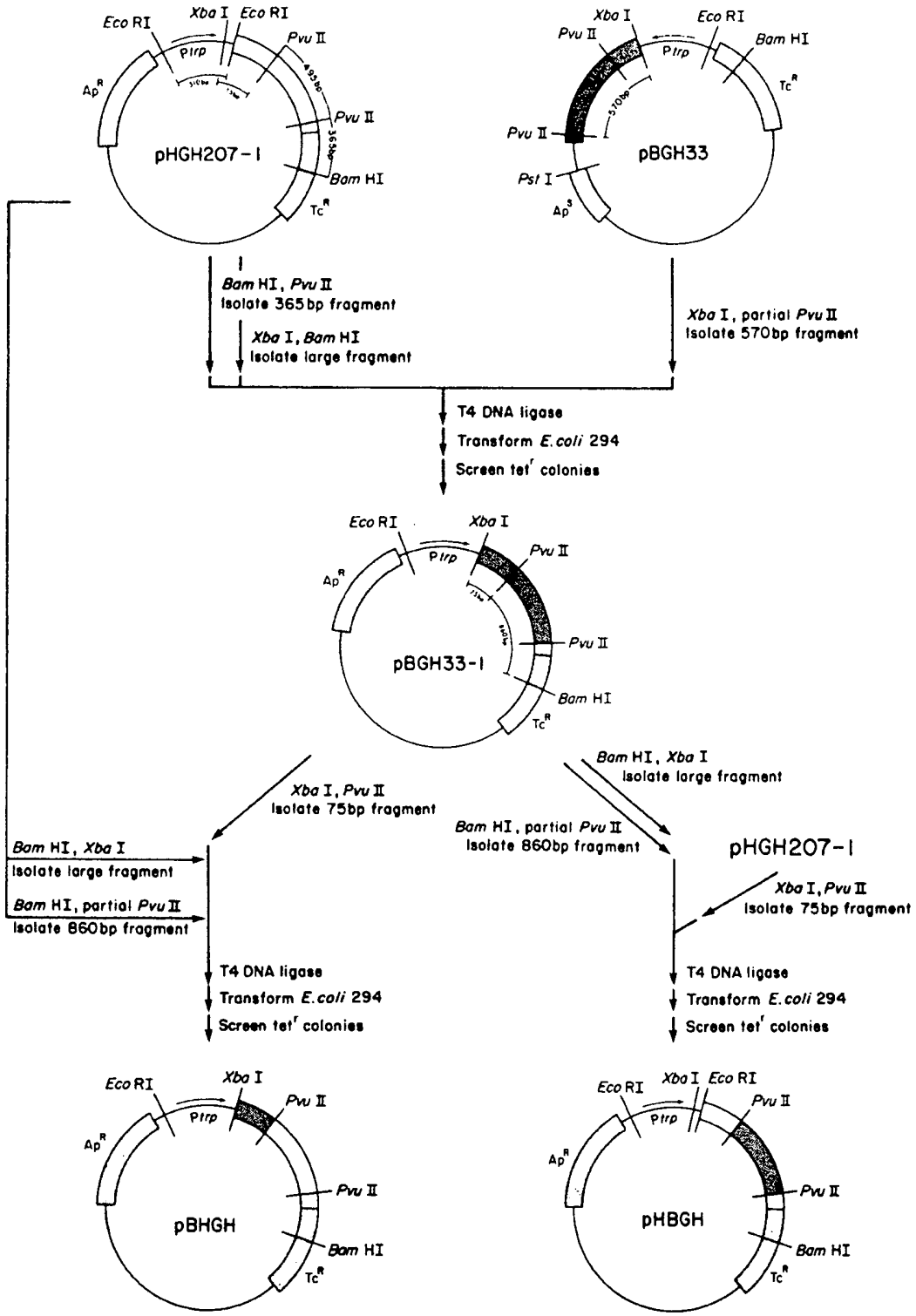
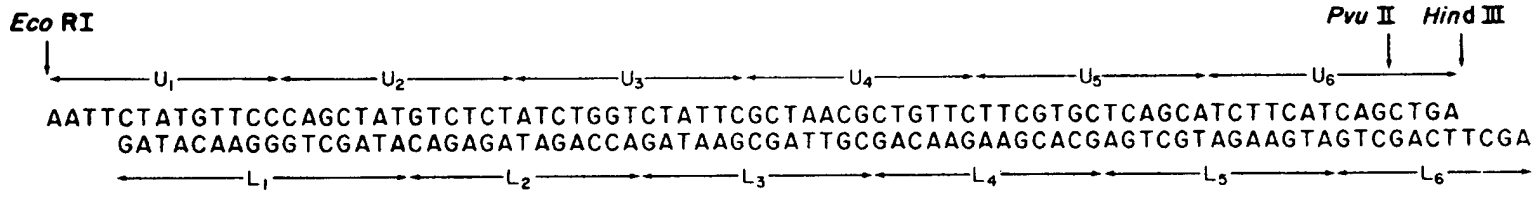

Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pHGH207-1
Pvu II
Bam HI
Tc^R
Xba I
Pvu II
Eco RI
Bam HI
Ptrp
Tc^R
Pvu II
pBGH33
Pst I
Ap^S
Bam HI, Pvu II
Isolate 365bp fragment
Xba I, Bam HI
Isolate large fragment
Xba I, partial Pvu II
Isolate 570bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Pvu II
Ptrp
Ap^R
pBGH33-1
Pvu II
Bam HI
Tc^R
Xba I, Pvu II
Isolate 75bp fragment
Bam HI, Xba I
Isolate large fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
Bam HI, Xba I
Isolate large fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
pHGH207-1
Xba I, Pvu II
Isolate 75bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Pvu II
Ptrp
Ap^R
pBHGH
Pvu II
Bam HI
Tc^R
Eco RI
Xba I
Eco RI
Pvu II
Ptrp
Ap^R
pHBGH
Pvu II
Bam HI
Tc^R

FIG. 5.



Mix Synthetic Oligonucleotides

$U_2-U_3-U_4-U_5-U_6$

$L_1-L_2-L_3-L_4-L_5$

T4 polynucleotide kinase

Phenol extract
Ethanol precipitate

Add U_1 and L_6
T4 DNA ligase

pBR322

15% polyacrylamide gel

Eco RI, *Hind* III
Isolate large fragment

Isolate 75bp band

T4 DNA ligase

Transform *E.coli* 294

Screen amp^r tet^s colonies

pBR322-01

FIG. 6.

EP 0 075 444 B1

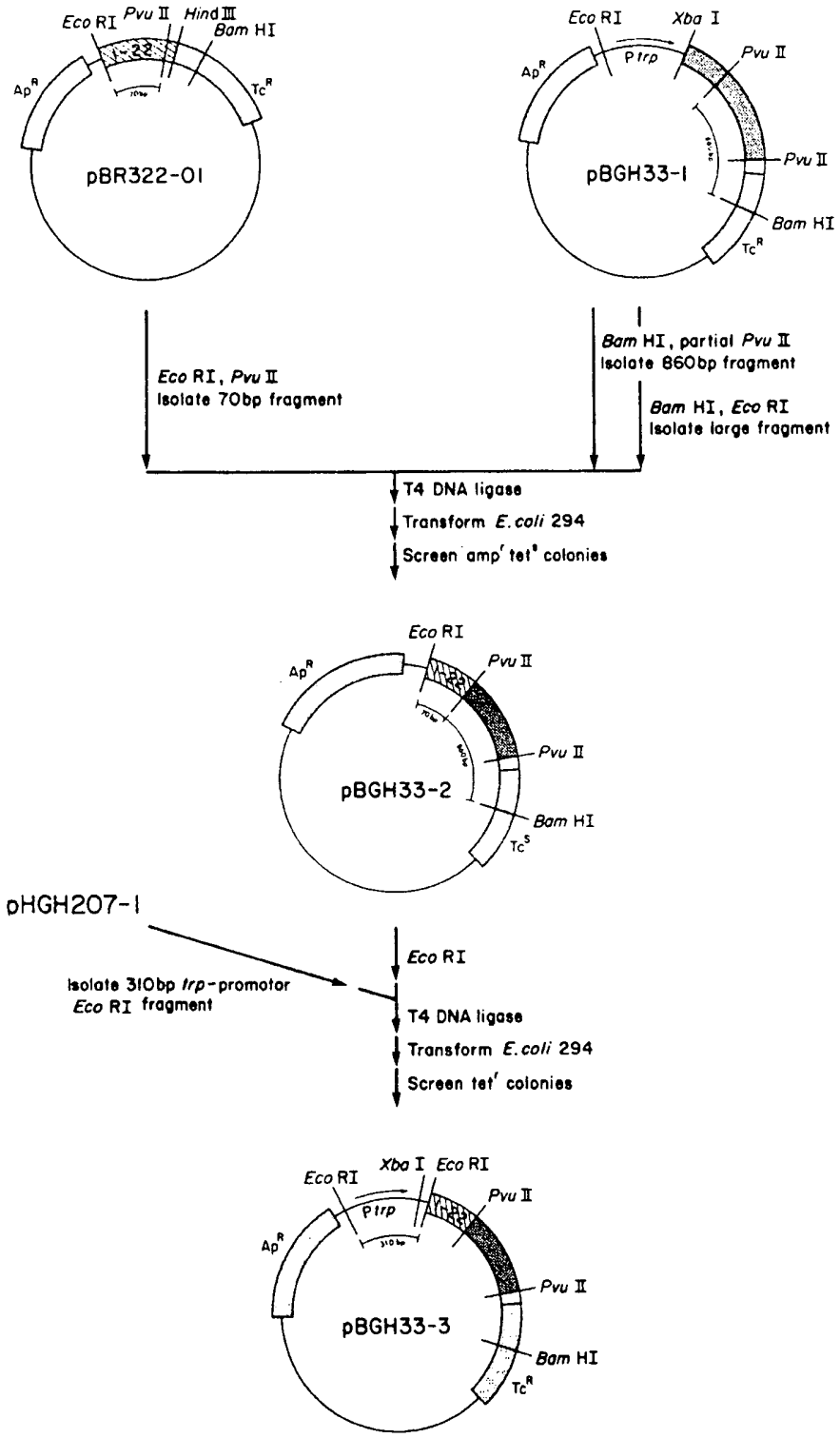

Eco RI
Pvu II
Hind III
Bam HI
Ap^R
Tc^R
pBR322-01
Eco RI
Xba I
P trp
Pvu II
Ap^R
pBGH33-1
Pvu II
Bam HI
Tc^R
Eco RI, Pvu II
Isolate 70bp fragment
Bam HI, partial Pvu II
Isolate 860bp fragment
Bam HI, Eco RI
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Screen amp^r tet^s colonies
Eco RI
Pvu II
Ap^R
pBGH33-2
Pvu II
Bam HI
Tc^S
pHGH207-1
Isolate 310bp trp-promotor
Eco RI fragment
Eco RI
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Eco RI
Xba I
Eco RI
P trp
Pvu II
Ap^R
pBGH33-3
Pvu II
Bam HI
Tc^R

FIG. 7.

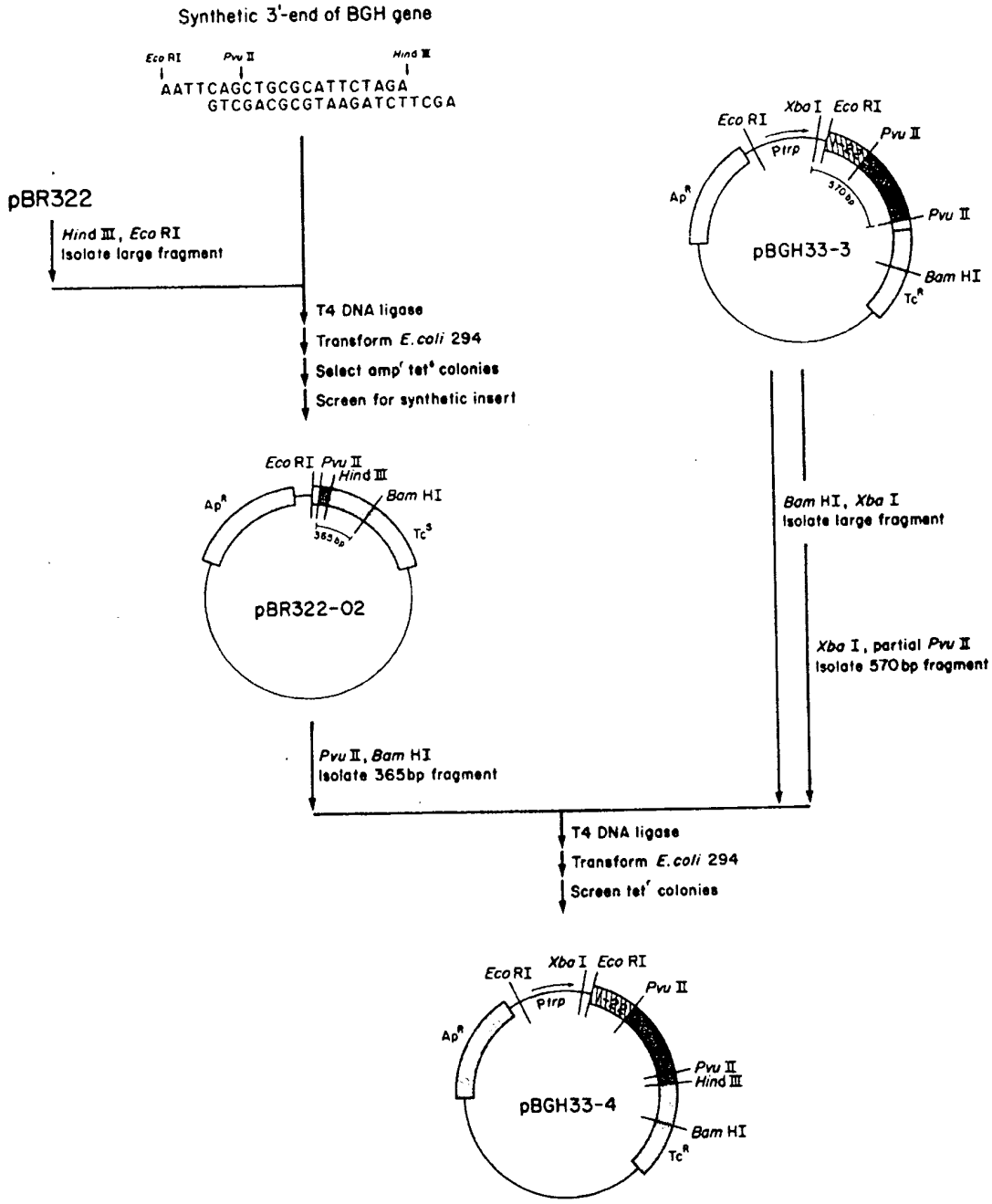
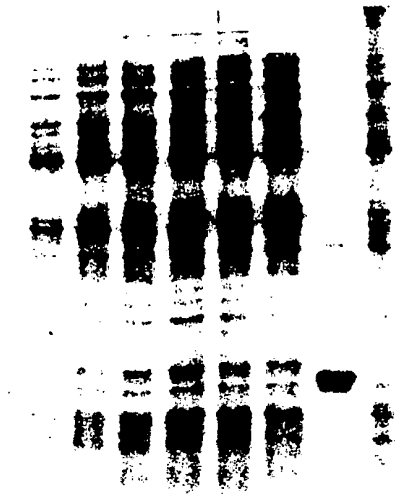
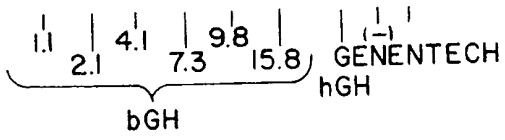

Synthetic 3'-end of BGH gene
Eco RI
Pvu II
Hind III
AATTCAGCTGCGCATTCTAGA
GTCGACGCGTAAGATCTTCGA
pBR322
Hind III, Eco RI
Isolate large fragment
T4 DNA ligase
Transform E.coli 294
Select amp^r tet^s colonies
Screen for synthetic insert
Eco RI Pvu II Hind III
Bam HI
Ap^R
Tc^S
365 bp
pBR322-02
Pvu II, Bam HI
Isolate 365bp fragment
Xba I Eco RI
Eco RI
Pvu II
Ptrp
Ap^R
570 bp
Pvu II
pBGH33-3
Bam HI
Tc^R
Bam HI, Xba I
Isolate large fragment
Xba I, partial Pvu II
Isolate 570bp fragment
T4 DNA ligase
Transform E.coli 294
Screen tet^r colonies
Xba I Eco RI
Eco RI
Pvu II
Ptrp
Ap^R
Pvu II
Hind III
pBGH33-4
Bam HI
Tc^R

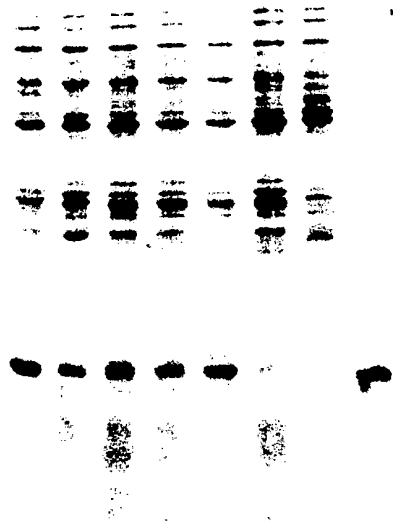
FIG. 8.

A





B



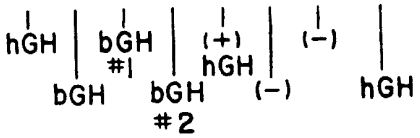


FIG. 9.

CIBC

CIBC WORLD MARKETS

Equity Research

February 20, 2003

Company Rating: *Sector Performer*

Company Update

Sector Weighting: *Overweight*

Johnson & Johnson

Rapamune Warning— Much Ado About Nothing

Pharmaceuticals

JNJ-NYSE (2/21/03)	**$51.92**
12-18 mo. Price Target	None
Key Indices: S&P 500, NYSE, Pharma, S&P 100	
3-5-Yr. EPS Gr. Rate (E):	12.0%
52-week Range	$65.89-$41.40
Shares Outstanding	3,011M
Float	3,007.7M shrs
Avg. Daily Trading Vol.	8,537,200
Market Capitalization	$156.3B
Dividend/Yield	$0.82/1.6%
Fiscal Year Ends	December
Book Value	8.05 per Shr
2003 ROE	23.8%
LT Debt	$2,217.00
Preferred	Nil
Common Equity	$24,233.00M
Convertible Available	Yes

- A "Dear Healthcare Provider" letter concerning Rapamune (Wyeth) was posted to the FDA's Web site late Thursday. The update warns of a serious pulmonary disorder occurring in lung transplant patients receiving treatment with Rapamune, tacrolimus and corticosteroids.

- We do not foresee a problem for JNJ's Cypher stent, which incorporates Rapamune. Cypher uses a small amount (180 mcg/stent) for local delivery while transplant patients need systemic therapy of 2-5 mg/day. This transplant drug regimen is not commonly seen in PCTA patients.

- JNJ shares have been weak on concerns regarding a potential delay of approval for Cypher. Although the visibility is low, we continue to expect approval in late 1Q03/early 2Q03. Weakness from this issue could provide investors with a buying opportunity.

- We continue to like JNJ's long-term prospects (Cypher, emphasis on drugs) but expect that near-term cracks in the drug business and possibility of approval delay for Cypher will contain valuation to $55 per share. We maintain a Sector Performer rating on the shares.

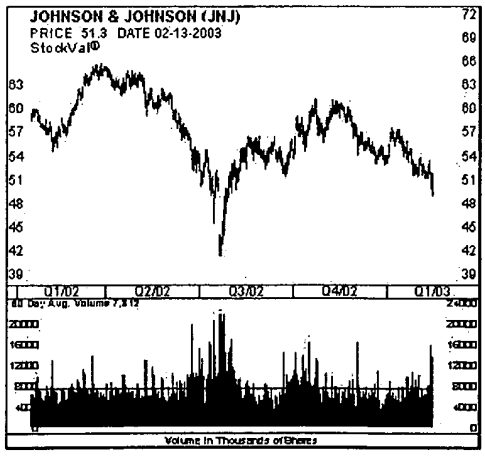
Company Description

A diversified healthcare company with strengths in pharmaceuticals, medical devices, and consumer health.

Earnings per Share	Prev	Current
2002A		$2.24A
2003E		$2.62E
2004E		$3.00E
P/E		
2002A		23.2x
2003E		19.8x
2004E		17.3x

3Q02 restated for Amgen litigation settlement

Stock Price Performance



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See "Legal Disclaimer" section at the end of this report for important disclosures, including potential conflicts of interest.

Investment Thesis

Johnson & Johnson is delivering solid results, as the company's increasing focus on higher margin businesses (pharmaceuticals, stents) propels EPS growth over the next few years. Pharmaceuticals (47% of total sales, but greater than 60% of profits) have been the key driver of sales and EPS growth over the past several years, but prospects have begun to moderate based on competitive pressures, including patent expirations, and the launch of competitor products. To address this, Johnson & Johnson is acquiring Scios, a biotechnology company, for $2.4 billion (announced February 10). The acquisition of Scios will bring Natrecor, a modest product for congestive heart failure, but importantly brings SCIO-469, a potential blockbuster for the rheumatoid arthritis.

Long-term Prospects Strong; Near-term Visibility Issues

Weakness in the pharmaceutical business is also likely to be offset by the market introduction of the Cypher drug-eluting stent, which should provide the bulk of the company's growth in 2003. Expectations for Cypher are high, but timing of the product's U.S. launch is uncertain, and this is a concern for investors. Timing of the Cypher launch is pegged for late 1Q03/early 2Q03, a timeline we had anticipated following receipt of a major deficiency letter from the FDA last fall, but perhaps slightly behind market expectations. As a result, expansion of Johnson & Johnson's P/E multiple, which is already at a significant premium to those of the S&P 500 (22%) and peers (16%), appears limited. This tempers our rating on the shares to a Sector Performer.

Investment Risks

Our investment thesis for Johnson & Johnson may not be realized if one or more of the following negative factors arise: material adverse events relating to the current investigations regarding Procrit/Eprex; a significant decline in the Procrit/Eprex franchise; delays in new product approvals (notably, the Cypher drug-eluting stent); unexpected capacity constraints, product recalls, or patent loss; and regulatory oversight. Even if none of the above negative factors does arise, our view on JNJ's prospects may still not be realized as a result of events pertaining to pharmaceutical industry and/or the broader market.

Exhibit 1. Sales and Earnings by Year

Johnson & Johnson — **Income Statement by Year (a)**

Year ended December ($ in millions)	2001A(b)	% Chg.	2002A(c)	% Chg.	2003E	% Chg.	2004E	% Chg.	2005E	% Chg.
Pharmaceuticals	$14,851	17.3%	$17,151	15.5%	$19,290	12.5%	$21,220	10.0%	$22,990	8.3%
Medical Devices & Diagnostics	11,146	8.4	12,583	12.9	13,770	9.4	15,700	14.0	17,620	12.2
Consumer Healthcare	6,320	(8.5)	6,564	3.9	7,365	12.2	7,705	4.6	7,965	3.4
Total sales	**$32,317**	8.3%	**$36,298**	12.3%	**$40,425**	11.4%	**$44,625**	10.4%	**$48,575**	8.9%
Cost of sales	9,581	7.6	10,447	9.0	11,570	10.7	12,575	8.7	13,550	7.8
Gross profit	$22,736	8.6%	$25,851	13.7%	$28,855	11.6%	$32,050	11.1%	$35,025	9.3%
Gross profit margin	*70.4%*		*71.2%*		*71.4%*		*71.8%*		*72.1%*	
SG&A	11,260	0.4	12,216	8.5	13,425	9.9	14,625	8.9	15,905	8.8
SG&A/sales	*34.8%*		*33.7%*		*33.2%*		*32.8%*		*32.7%*	
R&D	3,591	15.7	3,957	10.2	4,340	9.7	4,800	10.6	5,265	9.7
R&D/sales	*11.1%*		*10.9%*		*10.7%*		*10.8%*		*10.8%*	
Operating income	**$7,885**	19.2%	**$9,678**	22.7%	**$11,090**	14.6%	**$12,625**	13.8%	**$13,855**	9.7%
Operating margin	*24.4%*		*26.7%*		*27.4%*		*28.3%*		*28.5%*	
Interest & other, net	265	(7.3)	(113)	(142.6)	70	(161.9)	100	42.9	155	55.0
Pretax income	**$8,150**	18.1%	**$9,565**	17.4%	**$11,160**	16.7%	**$12,725**	14.0%	**$14,010**	10.1%
Taxes	2,230	17.2	2,725	22.2	3,249	19.2	3,690	13.6	4,063	10.1
Tax rate	*27.4%*		*28.5%*		*29.1%*		*29.0%*		*29.0%*	
Net income	**$5,920**	18.4%	**$6,840**	15.5%	**$7,911**	15.7%	**$9,035**	14.2%	**$9,947**	10.1%
Net margin	*18.3%*		*18.8%*		*19.6%*		*20.2%*		*20.5%*	
EPS, diluted	**$1.91**	17.5%	**$2.24**	17.3%	**$2.62**	17.1%	**$3.00**	14.3%	**$3.30**	10.1%
Diluted Shares outstanding	3,099.3	0.0	3,054.1	(1.5)	3,017.2	(1.2)	3,015.0	(0.1)	3,016.0	0.0

Source: Johnson & Johnson, CIBC World Markets

(a) 2002-03 include $0.04 in each year for a change in accounting for goodwill.

(b) 2001 excludes one-time after tax charges of $231 million for ALZA merger, and Inverness Medical and TERAMED acquisitions. Including charges, EPS $1.84.

(c) 2002 excludes one-time after tax charges of $189 million for Tibotec-Virco and Obtech Medical acquisitions. Including charges, EPS $2.18.

Exhibit 2. Product Sales by Year

Johnson & Johnson										Product Sales by Year
Year ended December ($ in millions)	2001A	% Chg.	2002A	% Chg.	2003E	% Chg.	2004E	% Chg.	2005E	% Chg.
Key Products										
Procrit	$3,430	*26.6%*	$4,269	*24.5%*	$4,800	*12.4%*	$5,175	*7.8%*	$5,460	*5.5%*
Risperdal	1,845	*15.1*	2,146	*16.3*	2,215	*3.2*	2,400	*8.4*	2,585	*7.7*
Remicade	721	*94.9*	1,297	*79.9*	1,745	*34.5*	2,155	*23.5*	2,515	*16.7*
Topamax	477	*54.4*	687	*44.0*	850	*23.7*	1,050	*23.5*	1,295	*23.3*
Cordis	1,344	*27.3*	1,641	*22.1*	2,350	*43.2*	3,500	*48.9*	4,690	*34.0*
Duragesic	$875	*33.4%*	$1,203	*37.5%*	$1,260	*4.7%*	$1,435	*13.9%*	$1,575	*9.8%*
Sporonox	603	*(0.2)*	568	*(5.8)*	525	*(7.6)*	485	*(7.6)*	450	*(7.2)*
Levaquin/Floxin	1,052	*(3.4)*	1,032	*(1.9)*	915	*(11.3)*	870	*(4.9)*	825	*(5.2)*
Oral Contraceptives	1,003	*4.9*	1,003	-	900	*(10.3)*	850	*(5.6)*	800	*(5.9)*
Ultram/Ultracet	601	*15.4*	442	*(26.5)*	350	*(20.8)*	300	*(14.3)*	250	*(16.7)*
Risperdal	1,845	*15.1*	2,146	*16.3*	2,215	*3.2*	2,400	*8.4*	2,585	*7.7*
Procrit	3,430	*26.6*	4,269	*24.5*	4,800	*12.4*	5,175	*7.8*	5,460	*5.5*
Remicade	721	*94.9*	1,297	*79.9*	1,745	*34.5*	2,155	*23.5*	2,515	*16.7*
Topamax	477	*54.4*	687	*44.0*	850	*23.7*	1,050	*23.5*	1,295	*23.3*
Aciphex	556	*102.2*	697	*25.4*	910	*30.6*	1,060	*16.5*	1,180	*11.3*
Other	2,278	*(9.7)*	2,122	*(6.8)*	2,750	*29.6*	3,000	*9.1*	3,250	*8.3*
Total Pharmaceuticals	$14,851	*17.3%*	$17,151	*15.5%*	$19,290	*12.5%*	$21,220	*10.0%*	$22,990	*8.3%*
Ethicon	$2,130	*14.0%*	$2,562	*20.3%*	$2,400	*(6.3%)*	$2,550	*6.3%*	$2,700	*5.9%*
Endo-Surgery	2,000	*36.1*	2,291	*14.6*	2,315	*1.0*	2,455	*6.0*	2,590	*5.5*
Cordis	1,344	*27.3*	1,641	*22.1*	2,350	*43.2*	3,500	*48.9*	4,690	*34.0*
Lifescan	1,095	*10.8*	1,342	*22.6*	1,400	*4.3*	1,540	*10.0*	1,630	*5.8*
Ortho-Clinical Diagnostics	1,022	*4.6*	1,094	*7.0*	1,125	*2.8*	1,175	*4.4*	1,225	*4.3*
DePuy	2,053	*13.1*	2,359	*14.9*	2,415	*2.4*	2,600	*7.7*	2,775	*6.7*
Vistakon	1,044	*1.2*	1,169	*12.0*	1,225	*4.8*	1,300	*6.1*	1,400	*7.7*
Other Professional	460	*360.0*	125	*(72.8)*	540	*332.0*	580	*7.4*	610	*5.2*
Total Medical Devices	$11,146	*8.4%*	$12,583	*12.9%*	$13,770	*9.4%*	$15,700	*14.0%*	$17,620	*12.2%*
Skin Care	$1,394	*(7.4%)*	$1,571	*12.7%*	$1,650	*5.0%*	$1,780	*7.9%*	$1,875	*5.3%*
McNeil	1,742	*(1.3)*	1,800	*3.3*	2,000	*11.1*	2,050	*2.5*	2,120	*3.4*
Baby & Kids Care	1,132	*(8.3)*	1,161	*2.6*	1,315	*13.3*	1,385	*5.3*	1,410	*1.8*
Women's Health	1,229	*(8.6)*	1,275	*3.7*	1,400	*9.8*	1,450	*3.6*	1,480	*2.1*
Other Consumer Products	823	*(22.1)*	757	*(8.0)*	1,000	*32.1*	1,040	*4.0*	1,080	*3.8*
Total Consumer Products	$6,320	*(8.5%)*	$6,564	*3.9%*	$7,365	*12.2%*	$7,705	*4.6%*	$7,965	*3.4%*
Total	**$32,317**	*8.3%*	**$36,298**	*12.3%*	**$40,425**	*11.4%*	**$44,625**	*10.4%*	**$48,575**	*8.9%*

Source: Johnson & Johnson, CIBC World Markets

Source: CIBC World Markets Corp.

Exhibit 3. Selected Product Pipeline

Johnson & Johnson* — **Selected Product Pipeline**

Drug	Therapeutic Category: Indication	Description	Stage of Development: US	Stage of Development: Europe	Comments
Risperdal Consta (a)	CNS: schizophrenia	5HT2/D2 antagonist depot	Not approvable	Filed	FDA meetings have taken place.
Aciphex (a)	Gastrointestinal: *h. pylori* eradication	Proton pump inhibitor	Filed	**	Filed January 2002.
Levaquin (a)	Infectious disease: nosocomial pneumonia	Quinolone antibiotic	Filed	Phase III	Filed December 2001.
Reminyl (a)	CNS: Alzheimer's disease (oral formulation)	Acetylcholinesterase inhibitor	Filed	Phase III	Filed December 2001.
Risperdal (a)	CNS: dementia (Alzheimer's related, oral formulation)	5HT2/D2 antagonist	Filed	Filed	Filed November 2001.
Risperdal (a)	CNS: bipolar disorder	5HT2/D2 antagonist	Filed	Filed	Filed December 2002.
Topamax (a)	CNS: migraine prophylaxis	Anti-epileptic	Filed	Phase III	Filed December 2002.
Topamax (a)	CNS: partial onset seizures (monotherapy)	Anti-epileptic	Phase III	Phase III	Filed November 2002.
Risperdal (a)	CNS: dementia (Alzheimer's related)	5HT2/D2 antagonist	Phase III	Filed	Trial being redone in US.
Remicade (a)	Autoimmune: Crohn's disease (fistulizing)	Monoclonal antibody	Phase III	Phase III	Developed by Centocor.
Remicade (a)	Autoimmune: juvenile or early rheumatoid arthritis	Monoclonal antibody	Phase III	Phase III	Developed by Centocor.
Remicade (a)	Autoimmune: psoriasis, psoriatic arthritis	Monoclonal antibody	Phase III	Phase III	Developed by Centocor.
Remicade (a)	Autoimmune: Ankylosing spondylitis	Monoclonal antibody	Phase III	Phase III	EU filing expected in late 2002.
Remicade (a)	Gastrointestinal: ulcerative colitis	Monoclonal antibody	Phase III	Phase III	Developed by Centocor.
Procrit (a)	Oncology: anemia in critically ill patients	Recombinant protein	Phase III	Phase III	Currenty dosed off label.
Procrit (a)	Oncology: Chemotherapy induced anemia (1X week)	Recombinant protein	Phase III	Phase III	Currenty dosed off label.
Levaquin (a)	Infectious disease: prostatitis	Quinolone antibiotic	Phase III	Phase III	
Levaquin (a)	Infectious disease: 750mg short course CAP	Quinolone antibiotic	Phase III	Phase III	
Doxil (a)	Oncology: multiple myeloma	Liposomal chemotherapeutic	Phase III	Phase III	Developed by ALZA.
E-trans (a)	CNS: acute post-operative pain	Electrotransdermal fentanyl	Phase III	Phase III	Filing expected in 2003.
Topamax (a)	Metabolic: obesity	Anti-epileptic	Phase III	Phase III	
Reminyl (a)	CNS: vascular and lewy body dementia	Acetylcholinesterase inhibitor	Phase III	Phase III	Licensed from Shire.
Reminyl (a)	CNS: mild cognitive demential	Acetylcholinesterase inhibitor	Phase III	Phase III	Licensed from Shire.
ReoPro/Retavase (a)	Cardiology: acute myocardial infarction	Thrombolytics	Phase III	Phase III	Co-marketed with Eli Lilly.
Ultracet (a)	CNS: chronic pain	Ultram + acetominophen	Phase III	Phase III	Marketed for acute pain.
Yondelis	Oncology: sarcomas, breast, prostate cancers	Transcription factor inhibitor	Phase II	Phase II	Developed by Zeltia.
Zarnestra	Oncology	Farnesyl transferase inhibitor	Phase II	Phase II	Failed Phase III for pancreatic cancer.
Oxycodone OROS	CNS: chronic pain	Once daily opiod	Phase II	Phase II	Phase II data expected in 2003.
Flexeril 5	CNS: muscle spasm	Once daily neuromuscular blocker	Phase II	Phase II	Developed by ALZA.
Concerta	Oncology: cancer fatigue	Once daily methyphenidate	Phase II	Phase II	Developed by ALZA.
Alprazolam OROS	CNS: anxiety	Once daily benzodiazepine	Phase II	Phase II	Developed by ALZA.
Dapoxetine	CNS: premature ejaculation	Selective serotonin reuptake inhibitor	Phase II	Phase II	Entering Phase III later this year.

Source: Johnson & Johnson, CIBC World Markets

* Does not include Scios developmental candidates.

** Not pursuing development in this territory.

(a) Line extension/new indication.

Exhibit 4.

Pharmaceutical Industry - EPS Estimates and Valuation

Company	Ticker Symbol	Rating	Price 02/20/03	52 Week High	52 Week Low	Annual Dividend	2002A (g)	2003E	2004E (h)	5 Yr % EPS Growth	P/E 2002A	P/E 2003E	P/E 2004E
AstraZeneca PLC	AZN	SU	34.44	52.04	28.00	0.70	1.84	1.57	NA	8	18.7	21.9	--
Bristol-Myers Squibb Co. (a)	BMY	SU	22.55	51.20	19.49	1.12	1.17	1.60	1.72	7	19.3	14.1	13.1
GlaxoSmithKline PLC (b)	GSK	SU	36.43	50.46	31.35	1.24	2.35	2.55	2.74	7	15.5	14.3	13.3
Johnson & Johnson	**JNJ**	**SP**	**51.92**	**65.89**	**41.40**	**0.82**	**2.24**	**2.62**	**3.00**	**12**	**23.2**	**19.8**	**17.3**
Eli Lilly & Co.	LLY	SP	56.85	81.09	43.75	1.34	2.55	2.55	2.90	10	22.3	22.3	19.6
Merck & Co. (c)	MRK	SU	53.38	64.50	38.50	1.44	3.14	3.45	3.78	8	17.0	15.5	14.1
Pfizer Inc.	PFE	SP	28.64	41.90	25.13	0.60	1.59	1.80	2.18	16	18.0	15.9	13.1
Pharmacia Inc. (d)	PHA	S	39.35	46.40	28.53	0.54	1.54	1.75	NA	10	25.6	22.5	--
Schering-Plough Corp.	SGP	SP	18.25	36.25	16.10	0.68	1.42	1.08	1.27	9	12.9	16.9	14.4
Wyeth (e)	WYE	SO	36.21	66.51	28.25	0.92	2.22	2.44	2.80	10	16.3	14.8	12.9
Share Weighted Average						**0.90**				**10.8**	**18.6**	**17.1**	**14.6**
S & P 500 (f)	SPX		837.10	1173.89	768.63	16.67	47.25	52.31	56.00		17.7	16.0	14.9

Company	Ticker Symbol	Stock Price Performance (% Change) 1 Mo.	3 Mo.	12 Mo.	YTD	Div. Yield	Net Debt ($ Bil)	Market Value ($ Bil)	EV to 2001A Sales	EBITDA	Gross Profit	Relative P/E (SPX) 2002A	2003E	2004E
AstraZeneca PLC	AZN	2.2	(12.5)	(30.1)	(1.9)	2.03	(2.867)	60.2	3.5	11.0	4.8	1.06	1.37	--
Bristol-Myers Squibb Co.	BMY	(10.3)	(15.4)	(50.3)	(2.6)	4.97	0.757	43.7	2.3	6.3	3.2	1.09	0.88	0.88
GlaxoSmithKline PLC	GSK	(8.8)	(7.3)	(27.0)	(2.7)	3.39	2.786	112.1	3.9	10.8	5.0	0.88	0.89	0.89
Johnson & Johnson	**JNJ**	**(9.2)**	**(13.5)**	**(9.4)**	**(3.3)**	**1.58**	**(5.190)**	**156.3**	**4.6**	**15.3**	**6.6**	**1.31**	**1.24**	**1.16**
Eli Lilly & Co.	LLY	(15.5)	(7.4)	(25.0)	(10.5)	2.36	(0.313)	63.9	5.5	14.2	6.8	1.26	1.39	1.31
Merck & Co.	MRK	(10.8)	(2.6)	(11.4)	(5.7)	2.70	5.579	120.9	2.7	10.0	6.8	0.96	0.97	0.94
Pfizer Inc.	PFE	(7.0)	(14.7)	(30.5)	(6.3)	2.09	0.259	178.8	5.6	14.3	6.6	1.02	0.99	0.88
Pharmacia Inc.	PHA	(6.8)	(13.5)	(0.9)	(5.9)	1.37	1.850	50.9	3.8	15.5	4.8	1.44	1.41	--
Schering-Plough Corp.	SGP	(20.5)	(15.2)	(47.3)	(17.8)	3.73	(2.039)	26.8	2.5	7.3	3.2	0.73	1.06	0.96
Wyeth	WYE	(5.3)	3.5	(42.9)	(3.2)	2.54	6.428	48.0	3.9	11.5	5.1	0.92	0.93	0.87
Share Weighted Average		**(8.6%)**	**(10.9%)**	**(26.9%)**	**(5.5%)**	**2.44**			**4.1**	**12.1**	**5.6**	**1.05**	**1.07**	**1.00**
S & P 500	SPX	(9.8)	(5.2)	(24.4)	(4.9)	1.99								

Rating Key: SO = Sector Outperformer; SP = Sector Performer; SU = Sector Underperformer; S = Suspended

The stock price performance results presented above cannot and should not be viewed as an indicator of future performance.

(a) EPS figures are pro forma for the acquisition of Du Pont Pharmaceuticals. (b) EPS translated @ $1.43 for 2002-03.

(c) The CIBC World Markets analyst(s) that covers this company also has a position in its securities.

(d) Pharmaceutical business only. (e) 2002 EPS includes $0.12 for a change in accounting of goodwill. (f) First Call estimates.

(g) CIBC estimate for Bristol-Myers Squibb and Pharmacia. (h) CIBC estimate for Pfizer is on a consolidated basis (includes Pharmacia).

Source: CIBC World Markets Corp. estimates, Company reports, Factset, ILX, First Call.

Our EPS estimates are shown below:

	1 Qtr.	2 Qtr.	3 Qtr.	4 Qtr.	Yearly
2002A Actual	$0.59A	$0.60A	$0.57A	$0.48A	$2.24A
2003E Current	$0.67E	$0.71E	$0.68E	$0.56E	$2.62E
2004E Current	--	--	--	--	$3.00E

Companies Mentioned In This Report

Stock Prices as of 2/20/03:

AstraZeneca PLC (AZN-NYSE $34.44 Sector Underperformer)
Eli Lilly & Co. (LLY-NYSE $56.85 Sector Performer)
Merck & Co. ((2a, 6, 9a)(MRK-NYSE $53.38 Sector Underperformer)
Pharmacia Corporation (PHA-NYSE $39.35 Suspended)
Wyeth (WYE-NYSE $36.21 Sector Outperformer)
Bristol-Myers Squibb (BMY-NYSE $22.55 Sector Underperformer)
GlaxoSmithKline ((2a, 9a)(GSK-NYSE $36.43 Sector Underperformer)
Pfizer Inc. (PFE-NYSE $28.64 Sector Performer)
Schering-Plough Corp. (SGP-NYSE $18.25 Sector Performer)

Key to Footnotes:

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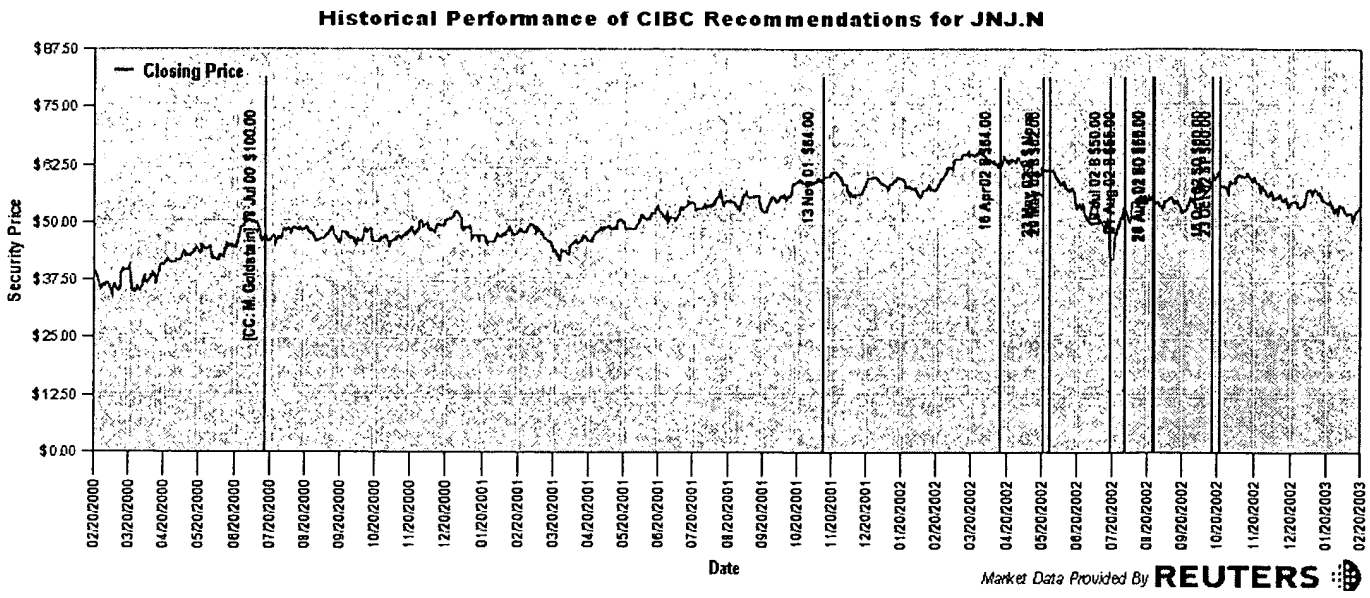
CIBCWM Price Chart



CIBCWM Stock Rating System

Abbreviation	Rating	Description
Company Ratings		
SO	Sector Outperformer	Stock is expected to outperform the sector during the next 12-18 months.
SP	Sector Performer	Stock is expected to perform in line with the sector during the next 12-18 months.
SU	Sector Underperformer	Stock is expected to underperform the sector during the next 12-18 months.
S	Suspended	Stock coverage is temporarily halted.
DR	Dropped	Stock coverage is discontinued.
NR	Not Rated	Stock is not covered by CIBCWM.
Company Ratings Prior To August 26th 2002		
SB	Strong Buy	Expected total return over 12 months of at least 25%.
B	Buy	Expected total return over 12 months of at least 15%.
H	Hold	Expected total return over 12 months of at least 0%-15%.
UP	Underperform	Expected negative total return over 12 months.
R	Restricted	Restricted
UR	Under Review	Under Review
Sector Weightings**		
O	Overweight	Sector is expected to outperform the broader market averages.
M	Market Weight	Sector is expected to equal the performance of the broader market averages.
U	Underweight	Sector is expected to underperform the broader market averages.
NA	None	Sector rating is not applicable.

**Broader market averages refer to the S&P 500 in the U.S. and S&P/TSX Composite in Canada.
"-S" indicates Speculative. An investment in this security involves a high amount of risk due to volatility and/or liquidity issues.
"CC" indicates Commencement of Coverage. The analyst named started covering the security on the date specified.

Ratings Distribution: CIBC World Markets Coverage Universe (as of 20 Feb 2003)	Count	Percent	Inv. Banking Relationships	Count	Percent
Sector Outperformer (Buy)	306	33.5%	Sector Outperformer (Buy)	191	62.4%
Sector Performer (Hold/Neutral)	403	44.1%	Sector Performer (Hold/Neutral)	221	54.8%
Sector Underperformer (Sell)	205	22.4%	Sector Underperformer (Sell)	89	43.4%
Ratings Distribution: Pharmaceuticals Coverage Universe (as of 20 Feb 2003)	**Count**	**Percent**	**Inv. Banking Relationships**	**Count**	**Percent**
Sector Outperformer (Buy)	3	21.4%	Sector Outperformer (Buy)	2	66.7%
Sector Performer (Hold/Neutral)	6	42.9%	Sector Performer (Hold/Neutral)	1	16.7%
Sector Underperformer (Sell)	5	35.7%	Sector Underperformer (Sell)	2	40.0%

Pharmaceuticals Sector includes the following tickers: AEGN, ALKS, ARDM, ATRX, AZN, BMY, DRRX, GSK, JNJ, LLY, MRK, NKTR, PFE, PHA, SGP, WYE.

Legal Disclaimers and Important Disclosure Footnotes

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SG COWEN

Johnson & Johnson
JNJ: $54
Rated 1 (S. Buy)

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January 22, 2003

A GOOD FINISH GIVES LOTS OF MOMENTUM HEADING INTO 2003

	Prior EPS FY-Dec	Current EPS FY-Dec	Revision	P/E	Quarterly EPS Q1	Q2	Q3	Q4
2001A		$1.91			$0.50	$0.51	$0.50	$0.39
2002A	$2.23	$2.24	+$0.01		$0.59	$0.60	$0.57	$0.48
2003E	$2.60	$2.62	+$0.02	21x	$0.68	$0.70	$0.67	$0.57
2004E	$2.95	$2.97	+$0.02	18x				

**GAAP EPS excluding one-time non-recurring charges.*

Conclusion:
J&J posted solid Q4 earnings that support the underlying strength of the company's key business franchises heading into 2003. Not surprisingly, investors aired concerns regarding slowing '03 pharmaceutical growth, however we remain comfortable with our 9-10% pharmaceutical forecast and our projections for both Procrit and Remicade. Cypher approval not likely to occur before late March, but we continue to believe launch timing supports our current top and bottom line forecast.

Key Points:
1. Q4:02 EPS of $0.48 +22% Y/Y, $0.01 ahead of consensus and good quality. Outlook remains strong.
2. Organic sales growth of 13% was 100bp above our expectation driven by MD&D upside. Pharmaceuticals performed relatively in line with our expectations.
3. Procrit/Eprex organic growth solid in U.S. (+21%), while Int'l down on PRCA (-5% ex-FX) as expected. Remicade very robust (+80%) driven by RA use and increasing Crohn's maintenance.
4. Cypher approval pegged for late Mar/early Apr, a modest slip from our mid-Mar. assumption. No change to our Cypher forecast (Q1 or '03) expected as impact from timing is less than $100MM.
5. Maintain organic top line growth assumption of 10.5% for '03. Lifting EPS by $0.02 to $2.62.

Investment Thesis:
We favor J&J for its robust top and bottom line growth prospects over the next several years, and believe our estimates have ample opportunity for upside. We view its growth fundamentals as the best in med tech; both the Pharmaceutical and Medical Devices & Diagnostics divisions are poised to deliver double-digit growth for the next several years. Also, J&J's impressive free cash flow gives the company unrivaled financial flexibility. Key franchises in the Pharmaceutical business – including Procrit, Remicade, and Topamax – should support sustained growth at the upper end of performance in the drug group. In the Medical Devices and Diagnostics division, numerous drivers, including the Cypher drug-coated stent and Lifescan diabetes products support an increase to double-digit growth over the next several years. Risks to our outlook include approval and launch timing of the Cypher stent, increased competition in the EPO market, and the potential for generic competition to key pharmaceutical franchises.

Discussion:
J&J reported Q4:02 EPS of $0.48, up 23% over the prior year - $0.01 ahead of the Street consensus and our estimate. EPS quality was solid, as the company posted upside to our sales ($9.4B, beat by $200MM) and gross margin assumption (20bp to 70.3%). R&D growth was modestly below our assumption, but still up 13% Y/Y bringing R&D spend for the full year just below management's $4B target. SG&A was up 8% modestly ahead of our estimate, but down 190bp as a percent of sales to 35.6%. Operating income was up 32% in the quarter, ahead of our expectation. Below the operating line, interest income was $12MM, below our model but reflective of reduced cash balances related to the company's stock buy back. Other income was $92MM (expense), heavier than our expectation. Pre-tax income was up 25% vs. our 23% expectation. Below the pretax line, J&J's effective tax rate was 25.6%, vs. 21.9% with the prior year. Shares were down 3% Y/Y, reflecting stock buy back. In summary, J&J delivered solid sales upside in the quarter and good quality EPS.

ONCE AGAIN - MD&D DROVE ORGANIC UPSIDE TO TOP LINE
Reported top line sales were $9,403MM, up 14.3% YY, ahead of our 11.9% growth expectation. Net of FX (a 1.5% gain) - organic sales growth was 12.8%, just ahead of our 12% expectation. Adjusted for comparisons against wholesale net de-stocking of pharmaceuticals in Q4:01, organic growth would have been 12.4% Y/Y. The Medical Devices and Diagnostics Division drove the lion's share of upside to our estimate posting organic growth of 15% vs our 11% expectation to report sales of $3,318MM (16% w/ FX benefit). Solid double-digit growth performances were posted by all seven subsegments - Ethicon (+10%), Ethicon Endo (+20%), Cordis (+23%), Depuy (+16%), Lifescan (+23%), Vistakon (+18% growth), and Ortho Clinical Diagnostics (+12%). Pharmaceutical growth was in line with our assumption at 16% with sales coming in at $4,435MM. Sales were positively impacted by a favorable comparison with wholesale de-stocking in Q4:01 ($120MM) - adjusting for this item, same store sales growth was estimated at 10% in line with our assumption. Pharmaceutical growth was led by Procrit (up 36%), Remicade (up 80%), Risperdal (up 23%), Duragesic (42%), Topamax (44%), and Aciphex (+26%). Levaquin/Floxin (-8%) was the only franchise to show sustainable downside to our forecast. Declines for Sporanox (-8%), Contraceptives (-9%) and Ultram (-65%) were in line with our thinking. The Consumer Division also performed ahead of our forecast, posting organic growth of 6.5% versus our 2-3% expectation to report sales of $1,650MM. Skincare (+18%) was an exceptionally strong performer in the quarter, as was Women's Health (+8%). McNeill Consumer (+4%) and Baby and Kids Care (+4%) were relatively in line.

J&J Q4:02 Sales by Division ($MM)

	Q4:02A	Q4:01A	%Δ	
Worldwide Total	9,403	8,225	14.3%	Op. growth 13%, 100bp ahead of our estimate.
Domestic	5,746	5,075	13.2%	Med Dev & Diag posted solid upside.
International	3,657	3,150	16.1%	Pharmaceutical in line with expectations.
Pharmaceutical	4,435	3,820	16.1%	Organic growth of 16%, in line with our estimate.
Domestic	3,088	2,651	16.5%	Organic growth adj. for stocking comparison was 10%.
International	1,347	1,169	15.2%	Procrit, Duragesic, Risperdal, Remicade & Topamax all strong.
Med. Dev. & Diag.	3,318	2,854	16.3%	Organic growth 15%, ahead of 11% expectation.
Domestic	1,770	1,574	12.5%	Every segment exceeded our forecast, and posted double digit growth.
International	1,548	1,280	20.9%	
Consumer	1,650	1,551	6.4%	Organic growth was 6.5% Y/Y.
Domestic	888	850	4.5%	Ahead of expectations from upside in Skin Care and Women's Health.
International	762	701	8.7%	

Source: Company reports

MODEST BOOST TO '03 ESTIMATES
Management backed the consensus EPS estimate of $2.62 and top line growth of 10-11% organic growth (12-13% with FX benefit). We maintained our 10.5% organic growth assumption and made a modest 50bp upward adjustment to our 2003 reported top line growth forecast to 11.5%, reflecting a FX benefit of 100bp

for the year. We forecast Pharmaceutical division growth of 10%, Medical Devices and Diagnostic Division growth of 18%, and Consumer growth of 4%. We made no change to our assumed 8-9% top line growth for the company in 2004. In 2004, we forecast Pharmaceutical division growth of 9-10%, Medical Devices and Diagnostic Division growth of 10%, and Consumer growth of 4%.

On the bottom line we raised our '03 EPS estimate by $0.02 to $2.62 – an estimate that we believe is achievable and has the potential to be exceeded. Our estimate assumes a Cypher launch occurs in mid March, although if approval were pushed out a few months, we would not anticipate needing to change our EPS estimate. We made no significant changes to our P&L assumptions for 2003, rather a few tweaks including modestly higher operating profit margin (GM and SG&A leverage), a higher assumed effective tax rate (29% vs. 28.5%), and lower other income/expense. For 2004, we forecast of EPS of $2.97 (+13%).

Pharmaceutical Detail

Pharmaceutical growth was in line with our assumption at 16% with sales coming in at $4,435MM. Sales were positively impacted by a favorable comparison with wholesale de-stocking in Q4:01 ($120MM) - adjusting for this item, same store sales growth was estimated at 10% in line with our assumption. Pharmaceutical growth was led by Procrit (up 36%), Remicade (up 80%), Risperdal (up 23%), Duragesic (42%), Topamax (44%), and Aciphex (+26%). Levaquin/Floxin (-8%) was the only franchise to show noticeable downside to our forecast. Declines for Sporanox (-8%), Contraceptives (-9%) and Ultram (-65%) were below our forecast, although declines for these businesses were more or less in line with our thinking.

Our growth assumption for the Pharmaceutical Division for 2003 is 10%, driven by double digit growth performances from Remicade (+37%), Risperdal (+10%), Duragesic (+22%), Topamax (+29%), Concerta (+26%) and Aciphex (+12%). We forecast Procrit/Eprex growth of 9%, which assumes U.S. growth of 10-11%. Detailed product discussions outlining Q4 results and our expectations for key product lines follow.

PROCRIT/EPREX: up 36% in the quarter to $1.096MM, $78MM ahead of our expectation (53% U.S.; 5% Int`l) driven by organic growth of 13% and boosted by a favorable comparison with the prior year (de-stocking). Organic growth in the U.S. adjusted for de-stocking was close to 21%, which we view as solid underlying growth in the face of increased competition from Aranesp. International sales of the product excluding FX was relatively in line with our projection – (5%) – as some erosion due to PRCA in chronic renal failure patients occurred. Our Procrit/Eprex sales forecast for 2003 is $4,648MM (+9%).

RISPERDAL: up 23% in the quarter ahead our estimate (19% U.S.; 30% Int`l) to $568MM. Underlying new prescription growth continues to support double-digit growth for the product despite increased competition. We believe the drug continues to gain broader use in the CNS market beyond its main schizophrenia indication including use in the treatment of bi polar mania. An sNDA for bi-polar as both monotherapy and adjunct to mood stabilizers was filed in December 2002, and Phase III trials for use of the drug Alzheimer's related psychoses are ongoing. The bi polar indication is currently approved in 13 OUS countries. Management was more communicative on the status of Risperdal Consta, noting that initial launches in OUS markets have gone well and that the product has received premium pricing to oral Risperdal. Although short of detailing a time line for resubmission in the U.S., we management's discussion of the product as a growth driver for the Risperdal franchise suggests that a late 2003 launch for the product in the U.S. is not unreasonable. Our growth assumption for Risperdal in 2003 is for sales to reach $2,355MM, up 10% Y/Y.

ANTI-INFECTIVES: down 8% in the quarter to $293MM, $52MM below our expectations (-7% U.S.; -13% Int`l). Sales were adversely impacted by a weak cough cold season and increased competition. Levaquin sales should be aided in 2003 by expanded labeling which differentiate the drug including antibiotic resistant strains of community acquired pneumonia, complicated skin infections, and nosocomial pneumonia. Other indications currently being pursued include short course therapy for pneumonia, bronchitis and sinusitus; prostatits; and acute otitis media (pediatric suspension). In 2003, we look for anti-infective sales of $1,129MM (+9%).

CONTRACEPTIVES: sales down 9% in the quarter to $239MM, $44MM below our expectations (-9% U.S.; -3% Int'l). Sales were negatively impacted by the introduction of generic Ortho Cyclen in late September, and likely some fall off from initial stocking of both Ortho Evra and Ortho Trio Cyclen Loa in Q3. We forecast contraceptive sales of $850MM (15%) in 2003. In addition to Ortho Cyclen, generics to Ortho Ovum 7-7-7 were launched in January and generics of Ortho Tri Cyclen are set for September 2003, although a pediatric extension could add 3 additional months of exclusivity. Growth potential for the franchise will rely on the success of the new Ortho products – Evra and TriCyclen Lo. On a positive note, prescription growth for Ortho Evra has accelerated significantly since August – with total prescriptions growing from 15,000 to over 100,000 in early January, and new prescription growth remaining robust. The current Rx run rate exiting 2002 is over $120MM in sales which is ahead of our expectations and puts Evra in a solid number 2 spot with the Ortho Contraceptives franchise heading into 2003. While the success of the product is not likely to be enough to stave off declines for the franchise in 2003 or 2004, the recent prescription acceleration gives us increased confidence in our estimates for the Contraceptive franchise.

DURAGESIC: up 42% to $320MM in the quarter, $34MM ahead of our expectations (42% U.S.; 41% Int'l). J&J continues to have good success with the product which is a transdermal patch for chronic pain and in our view, the product will continue to benefit from rapid expansion of the pain market. We raised our sales forecast for 2003 to $1,465MM up 22% Y/Y. Generic competition for Duragesic is expected in 2004, however, J&J has two alternative products to help defend the franchise. First is E-transfenta, an electronically mediated transdermal Fentanyl product for the post-operative pain market, which is in late stages of phase III clinical trials, and an NDA is expected to be filed in 2003. Second, is an improved lick-proof version of Duragesic, which is described to curb abuse potential, that is in phase II trials.

REMICADE: Q4 Remicade sales were up 80% over the prior year to $379MM, outpacing our forecast by $67MM. Sales upside was driven by strong international performance, driven in part by the launch of the product in Japan for Crohn's disease, and solid growth in the U.S. of 69% fueled by RA and the expanded label for maintenance Crohn's indications. We forecast 2003 growth of 37%, and forecast revenue of $1,782MM which we believe to be an above consensus forecast. There has been continued concern that Remicade sales growth will slow in 2003 from increased competition in the RA market from Humira and the re-launch of Enbrel. We believe our assumptions for Remicade in RA are reasonable in the face of increased competition, and look for new opportunities beyond RA - particularly Crohn's maintenance – to sustain solid growth for the product going forward well ahead of consensus views. In addition to Crohn's, we see solid opportunities for the drug in 2003 in expanded indications for maintenance fistulizing Crohn's (currently under priority review), as well as ulcerative colitis (Phase III) and psoriatic arthritis (Phase III) for which the drug has received limited Medicare and private pay reimbursement. The drug is also in Phase III trials for ankylosing spondylitis, juvenile RA, early RA and in pivotal Phase II/III studies for psoriasis.

ANALGESICS (ULTRAM/ULTRACET): down 65% in the quarter to $61MM, $10MM below our estimate. The severe decline was due to generic substitution. We estimate that Ultracet, the combination Ultram-Tylenol product will account for the lion's share of remaining analgesia sales – in excess of 80% in 2003. The company continues to pursue an expanded chronic pain management indication for Ultracet. We forecast 2003 sales of $230MM, -48% Y/Y which reflects the impact of directly substitutable generics in H1 and easing declines on comparisons in H2.

TOPAMAX: Sales in the quarter of $206MM, +45% Y/Y modestly below our forecast but still a solid performance. The anti-seizure drug is gaining increasing off-label use for bi-polar, migraine, and obesity. In late 2002, J&J filed sNDAs for the drug in monotherapy treatment of epilepsy and migraine prophylaxis. Development programs in bi polar, eating disorders and obesity are ongoing. The most recent new prescription growth posted has re-accelerated to greater than 60% Y/Y growth. In 2003 we expect revenue of $887MM, up 29% Y/Y, which assumes continued off-label gains driven by ongoing trial work.

ACIPHEX/PARIET WAS A SOLID PERFORMER (proton pump inhibitor for reflux/ulcers): Aciphex sales in the quarter were $183MM, up 26% Y/Y in line with our expectations. New prescription share has been relatively stable at 9% despite new competition, although we believe growth prospects will be muted in 2003 due to generic Prilosec competition. We believe the market growth the proton pump inhibitor market will be up the mid-teens for 2003, and look for Aciphex to post sales of $780MM (12% growth).

NO BREAK OUT OF REMINYL SALES, BUT LIKELY A CONTRIBUTOR (acetylcholinesterase inhibitor for Alzheimers): Although no sales data was shared by management, we believe Q4 sales of Reminyl were a modest contributor to sales growth. We continue to estimate the current market potential for Reminyl in Alzheimer's as a modest $300MM (our assumption is that no differentiating language in the U.S. label relegates the product to "me too" status vs. Exelon and Aricept). We are however, excited about additional development efforts for the drug which have the potential to significantly boost the product's market opportunity including clinical work in mixed and vascular dementia and an extended release formulation of the drug. Our 2003 forecast calls for $195MM in sales, up from $150MM in 2002.

Medical Devices & Diagnostics Detail

The Medical Devices and Diagnostics Division drove the lion's share of upside to our estimate posting organic growth of 15% vs our 11% expectation to report sales of $3,318MM (16% w/ FX benefit). U.S. sales were up 13%, and international sales were up 17% excluding currency. Results were ahead of expectations in all business segments Solid double-digit growth performances were posted by all seven subsegments - Ethicon (+10%), Ethicon Endo (+20%), Cordis (+23%), Depuy (+16%), Lifescan (+23%), Vistakon (+18% growth), and Ortho Clinical Diagnostics (+12%).

Our growth assumption for the Medical Devices & Diagnostics Division for 2003 is 18%, driven by ramping Cordis sales post-Cypher launch (+82%), and double digit sales growth from Lifescan (+11%), and high single digit sales growth from Ethicon Endo (+9%), Depuy (+8%) and Vistakon (+8%). Detailed product discussions outlining Q4 results and our expectations for key product lines follow.

CORDIS: sales were up 23% worldwide to $449MM (13% U.S.; 35% Int`l), modestly ahead of our expectation. U.S. sales were driven by the coronary stent business – particularly Hepacoat BX Velocity Rx stent sales. Because of new competition (BSX Express), management estimated U.S. coronary stent dollar share in the quarter down 400bp from Q3 to 30%. J&J dollar share for the quarter in Europe was estimated at 40%, up from 35% in Q3. Endovascular sales remained strong, up 13% Y/Y led by sales of new products including the Smarter, Precise and Genesis stents. We forecast 2003 sales of $2,990MM, +82% Y/Y which reflects the late March launch of the Cypher drug coated stent in the U.S.

LIFESCAN: sales growth ahead of our expectation at 22% in the quarter over the prior year to $213MM (16% U.S.; 33% Int`l). Sales continue to be driven by share gains of the company's flagship One Touch Ultra product. Growth was aided 200bp ($6MM) by the addition of the Can-Am/generic business acquired from Inverness Medical. We project 2003 growth of 11%, and forecast sales of $1,490MM. We note that during Q4, the company divested the Can Am business acquired from Inverness and has discontinued the Inverness private label business, which will negatively impact growth for the next several quarters (as we have modeled).

DEPUY: Sales were up a solid 16% to $623MM (U.S. 14%, Int`l 18%), ahead of our expectations driven by strong U.S. and international sales. Growth was driven by the spinal business, which was up 21%. The joint

reconstruction business also posted strong sales growth in the double-digit range fueled by a move to cementless Summit hips and Pinnacle cup and the success of the company's PFC knee system. We forecast 2003 sales o f $2,544MM, up 8% Y/Y.

ETHICON: Sales up 10% in the quarter to $669MM (8% U.S.; 12% Int`l), ahead of our projection. Growth drivers in the U.S. were the Gynecare and Mitek business units as well as positive contributions from the base wound care business worldwide. We look for 7% sales growth in 2003 to $2,744MM.

ETHICON ENDO: Sales up 20% in the quarter to $625MM (19% U.S.; 20% Int`l), ahead of our expectation. In the core endoscopy and mechanical segment, endocutters used in bariatric surgery contributed to solid gains. We forecast 2003 sales of $2,492MM, up 9% Y/Y.

VISTAKON: Sales up 18% Y/Y at $298MM (2% U.S., 29% Int`l) above our expectations driven by international sales. U.S. sales moderated despite positive contributions from a new line of color contact lenses. International sales were driven by sales of one-day Acuvue lens in Japan. We forecast sales of $1,262MM in 2003, up 8% Y/Y.

ORTHO CLINICAL DIAGNOSTICS: Sales up 12% to $281MM (5% U.S., 19% Int`l) ahead of our expectation. Growth was driven by pull through of infectious disease assays (Hep C) on the company's Eci immunoassay platform, continued strong growth in automated blood grouping and typing and blood screening platforms, and RhoGam product sales. We forecast 2003 sales of $1,156MM, up 6% Y/Y.

Consumer Detail

The Consumer Division also performed ahead of our forecast, posting organic growth of 6.5% versus our 2-3% expectation to report sales of $1,650MM. Skincare (+18%) was an exceptionally strong performer in the quarter, as was Women's Health (+8%). McNeill Consumer (+4%) and Baby and Kids Care (+4%) were relatively in line.

Regarding 2003, our growth expectation for the Consumer division is 4% to $6,796MM. We look for Skin Care to post high single-digit gains (+8%) but look for low single digit performance from Women's Health (+1%), McNeill Consumer (+3%) and Baby and Kids Care (+3%).

Estimated Quarterly Revenue ($MM)

	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1E	Q2 E	Q3 E	Q4 E	Year
Worldwide															
Med. Device & Diag.	2,735	2,785	2,772	2,854	11,146	2,958	3,166	3,141	3,318	12,583	3,340	3,771	3,734	3,947	14,792
Pharmaceutical	3,490	3,864	3,678	3,820	14,852	4,181	4,258	4,277	4,435	17,151	4,573	4,643	4,745	4,936	18,898
Consumer	1,630	1,530	1,609	1,551	6,320	1,604	1,649	1,661	1,650	6,564	1,687	1,711	1,704	1,693	6,796
WW Total	7,855	8,179	8,059	8,225	32,318	8,743	9,073	9,079	9,403	36,298	9,601	10,125	10,183	10,577	40,486
U.S.															
Med. Device & Diag.	1,463	1,530	1,569	1,574	6,136	1,663	1,758	1,740	1,770	6,931	1,858	2,186	2,230	2,285	8,560
Pharmaceutical	2,356	2,722	2,512	2,651	10,241	2,958	2,934	2,939	3,088	11,919	3,218	3,173	3,261	3,441	13,094
Consumer	896	807	896	850	3,449	900	907	910	888	3,605	929	935	936	912	3,712
U.S. Total	4,715	5,059	4,977	5,075	19,826	5,521	5,599	5,589	5,746	22,455	6,005	6,294	6,428	6,639	25,366
International															
Med. Device & Diag.	1,272	1,255	1,203	1,280	5,010	1,295	1,408	1,401	1,548	5,652	1,482	1,585	1,503	1,662	6,232
Pharmaceutical	1,134	1,142	1,166	1,169	4,611	1,223	1,324	1,338	1,347	5,232	1,355	1,471	1,483	1,495	5,804
Consumer	735	723	713	701	2,872	704	742	751	762	2,959	758	776	768	781	3,084
Int. Total	3,141	3,120	3,082	3,150	12,493	3,222	3,474	3,490	3,657	13,843	3,595	3,832	3,755	3,938	15,120
Growth Analysis															
Worldwide															
Med. Device & Diag.						8.2%	13.7%	13.3%	16.3%	12.9%	12.9%	19.1%	18.9%	19.0%	17.6%
Pharmaceutical						19.8%	10.2%	16.3%	16.1%	15.5%	9.4%	9.0%	10.9%	11.3%	10.2%
Consumer						-1.6%	7.8%	3.2%	6.4%	3.9%	5.2%	3.8%	2.6%	2.6%	3.5%
WW Total						11.3%	10.9%	12.7%	14.3%	12.3%	9.8%	11.6%	12.2%	12.5%	11.5%
U.S.															
Med. Device & Diag.						13.7%	14.9%	10.9%	12.5%	13.0%	11.7%	24.3%	28.2%	29.1%	23.5%
Pharmaceutical						25.6%	7.8%	17.0%	16.5%	16.4%	8.8%	8.1%	11.0%	11.4%	9.9%
Consumer						0.5%	12.4%	1.6%	4.5%	4.5%	3.2%	3.1%	2.9%	2.7%	3.0%
U.S. Total						17.1%	10.7%	12.3%	13.2%	13.3%	8.8%	12.4%	15.0%	15.5%	13.0%
International															
Med. Device & Diag.						1.8%	12.2%	16.5%	20.9%	12.8%	14.4%	12.6%	7.3%	7.4%	10.3%
Pharmaceutical						7.8%	15.9%	14.8%	15.2%	13.5%	10.8%	11.1%	10.9%	11.0%	10.9%
Consumer						-4.2%	2.6%	5.3%	8.7%	3.0%	7.7%	4.6%	2.2%	2.5%	4.2%
Int. Total						2.6%	11.3%	13.2%	16.1%	10.8%	11.6%	10.3%	7.6%	7.7%	9.2%

Johnson & Johnson Estimated Quarterly Profit and Loss Statement ($MM)

	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1E	Q2 E	Q3 E	Q4 E	Year
Total Revenue	7,855	8,179	8,058	8,225	32,317	8,743	9,073	9,079	9,403	36,298	9,601	10,125	10,183	10,577	40,486
Cost of Goods Sold	2,311	2,372	2,396	2,502	9,581	2,457	2,582	2,611	2,797	10,447	2,689	2,841	2,898	3,093	11,521
Gross Profit	5,544	5,807	5,662	5,723	22,736	6,286	6,491	6,468	6,606	25,851	6,912	7,284	7,285	7,484	28,965
R&D	759	829	899	1,104	3,591	831	932	952	1,242	3,957	961	1,050	1,094	1,422	4,527
SG&A	2,666	2,802	2,703	3,089	11,260	2,843	3,017	3,006	3,350	12,216	3,064	3,286	3,351	3,673	13,374
Operating Income	2,119	2,176	2,060	1,530	7,885	2,612	2,542	2,510	2,014	9,678	2,887	2,948	2,840	2,388	11,063
Interest	(92)	(70)	(67)	(74)	(303)	(42)	(30)	(12)	(12)	(96)	(12)	(20)	(25)	(30)	(87)
Other	(6)	15	(18)	55	46	33	(45)	129	92	209	0	0	0	0	0
Pretax Income	2,217	2,231	2,145	1,549	8,142	2,621	2,617	2,393	1,934	9,565	2,899	2,968	2,865	2,418	11,150
Taxes	665	647	579	339	2,230	787	774	668	496	2,725	841	861	831	701	3,234
Net Income	1,552	1,584	1,566	1,210	5,912	1,834	1,843	1,725	1,438	6,840	2,058	2,107	2,034	1,717	7,917
Net Interest Add Back*	9	9	0	0	18	0	0	0	0	0	0	0	0	0	0
Adj. Net Income	1,561	1,593	1,566	1,210	5,930	1,834	1,843	1,725	1,438	6,840	2,058	2,107	2,034	1,717	7,917
Shares (MM)	3,107	3,111	3,111	3,121	3,112	3,115	3,069	3,027	3,024	3,059	3,025	3,026	3,026	3,027	3,026
Earnings Per Share	$0.50	$0.51	$0.50	$0.39	$1.91	$0.59	$0.60	$0.57	$0.48	$2.24	$0.68	$0.70	$0.67	$0.57	$2.62
Margin Analysis															
Gross Profit	70.6%	71.0%	70.3%	69.6%	70.4%	71.9%	71.5%	71.2%	70.3%	71.2%	72.0%	71.9%	71.5%	70.8%	71.5%
R&D	9.7%	10.1%	11.2%	13.4%	11.1%	9.5%	10.3%	10.5%	13.2%	10.9%	10.0%	10.4%	10.7%	13.4%	11.2%
SG&A	33.9%	34.3%	33.5%	37.6%	34.8%	32.5%	33.3%	33.1%	35.6%	33.7%	31.9%	32.5%	32.9%	34.7%	33.0%
Operating Income	27.0%	26.6%	25.6%	18.6%	24.4%	29.9%	28.0%	27.6%	21.4%	26.7%	30.1%	29.1%	27.9%	22.6%	27.3%
Pretax Income	28.2%	27.3%	26.6%	18.8%	25.2%	30.0%	28.8%	26.4%	20.6%	26.4%	30.2%	29.3%	28.1%	22.9%	27.5%
Net Income	19.8%	19.4%	19.4%	14.7%	18.3%	21.0%	20.3%	19.0%	15.3%	18.8%	21.4%	20.8%	20.0%	16.2%	19.6%
Tax Rate	30.0%	29.0%	27.0%	21.9%	27.4%	30.0%	29.6%	27.9%	25.6%	28.5%	29.0%	29.0%	29.0%	29.0%	29.0%
Growth Analysis															
Total Revenue						11.3%	10.9%	12.7%	14.3%	12.3%	9.8%	11.6%	12.2%	12.5%	11.5%
Gross Profit						13.4%	11.8%	14.2%	15.4%	13.7%	10.0%	12.2%	12.6%	13.3%	12.0%
R&D	12.1%	16.3%	21.7%	13.1%	15.7%	9.5%	12.4%	5.9%	12.5%	10.2%	15.6%	12.7%	14.9%	14.5%	14.4%
SG&A						6.6%	7.7%	11.2%	8.4%	8.5%	7.8%	8.9%	11.5%	9.6%	9.5%
Operating Income	15.0%	16.6%	18.3%	31.3%	19.2%	23.2%	16.8%	21.8%	31.6%	22.7%	10.5%	16.0%	13.1%	18.6%	14.3%
Pretax Income	15.8%	16.6%	17.0%	24.9%	18.0%	18.2%	17.3%	11.6%	24.9%	17.5%	10.6%	13.4%	19.7%	25.0%	16.6%
Net Income	16.6%	16.2%	18.3%	23.3%	18.3%	18.1%	16.4%	10.2%	18.8%	15.7%	12.3%	14.3%	17.9%	19.4%	15.7%
EPS	14.2%	15.4%	17.6%	22.0%	17.0%	17.1%	17.3%	13.2%	22.7%	17.4%	15.6%	16.0%	17.9%	19.3%	17.0%

Johnson & Johnson Estimated Annual Profit and Loss Statement ($MM)

	2001	2002	2003E	2004E	2005E	2006E	01-06E Annual Growth Rate
Total Revenues	32,317	36,298	40,486	43,922	47,580	51,346	9.7%
Cost of Goods Sold	9,581	10,447	11,521	12,323	13,206	14,098	
Gross Profit	22,736	25,851	28,965	31,599	34,373	37,249	10.4%
R&D	3,591	3,957	4,527	5,022	5,559	5,947	
SG&A	11,260	12,216	13,374	14,150	14,909	15,627	
Operating Income	7,885	9,678	11,063	12,428	13,906	15,674	14.7%
Interest, Net	(303)	(96)	(87)	(240)	(400)	(550)	
Other, Net	46	209	0	0	0	0	
Pretax Income	8,142	9,565	11,150	12,668	14,306	16,224	14.8%
Taxes	2,230	2,725	3,234	3,674	4,149	4,705	
Net Income	5,912	6,840	7,917	8,994	10,157	11,519	14.3%
Net Int. Add Back	18	0	0	0	0	0	
Adj. Net Income	5,930	6,840	7,917	8,994	10,157	11,519	14.2%
Shares (MM)	3,112	3,059	3,026	3,026	3,026	3,026	
Earnings Per Share	$1.91	$2.24	$2.62	$2.97	$3.36	$3.81	14.8%
Growth Analysis							
Total Revenues	8.3%	12.3%	11.5%	8.5%	8.3%	7.9%	
Gross Profit	8.6%	13.7%	12.0%	9.1%	8.8%	8.4%	
R&D	15.7%	10.2%	14.4%	10.9%	10.7%	7.0%	
SG&A	0.4%	8.5%	9.5%	5.8%	5.4%	4.8%	
Operating Income	19.2%	22.7%	14.3%	12.3%	11.9%	12.7%	
Pretax Income	18.0%	17.5%	16.6%	13.6%	12.9%	13.4%	
Net Income	18.3%	15.7%	15.7%	13.6%	12.9%	13.4%	
Earnings Per Share	17.0%	17.4%	17.0%	13.6%	12.9%	13.4%	
Margin Analysis							
Gross Margin	70.4%	71.2%	71.5%	71.9%	72.2%	72.5%	
R&D	11.1%	10.9%	11.2%	11.4%	11.7%	11.6%	
SG&A	34.8%	33.7%	33.0%	32.2%	31.3%	30.4%	
Operating Income	24.4%	26.7%	27.3%	28.3%	29.2%	30.5%	
Pretax Income	25.2%	26.4%	27.5%	28.8%	30.1%	31.6%	
Net Income	18.3%	18.8%	19.6%	20.5%	21.3%	22.4%	
Tax Rate	27.4%	28.5%	29.0%	29.0%	29.0%	29.0%	

Estimated Annual Cash Flow Statement ($MM)

	2001	2002E	2003E	2004E	2005E	2006E
Beginning Cash & Equivalents	4,278	3,758	4,167	6,859	10,215	14,167
Operating Transactions						
Net Income	5,668	6,840	7,917	8,994	10,157	11,519
Non-Cash Charges	1,703	1,782	1,979	2,197	2,439	2,707
Depreciation & Amortization	1,605	1,782	1,978	2,195	2,437	2,705
Other Non-Cash Charges	98	0	1	2	2	2
Cash From Operations	7,371	8,621	9,895	11,191	12,595	14,226
Accounts Receivable	(258)	(534)	(555)	(442)	(465)	(473)
Inventories	(167)	(354)	(346)	(269)	(282)	(285)
Other Current Assets	(270)	(318)	(328)	(255)	(267)	(269)
Current/Non-current Liabilities	2,188	921	969	795	846	872
Net Operating Cash Flow	8,864	8,336	9,635	11,020	12,427	14,070
Capital Expenditures	(1,731)	(2,100)	(2,205)	(2,315)	(2,431)	(2,553)
Dividends	(2,047)	(2,400)	(2,760)	(3,174)	(3,650)	(4,198)
Free Cash Flow	5,086	3,836	4,670	5,531	6,346	7,320
Asset sales	163	0	0	0	0	0
Acquisitions	(225)	(466)	0	0	0	0
Other, Net	(2,300)	0	0	0	0	0
Net From Operating Trans.	2,724	3,370	4,670	5,531	6,346	7,320
Non-Operating Transactions						
Total Debt	(1,148)	1,974	0	0	0	0
Share Repurchases	(2,570)	(5,500)	(2,600)	(2,860)	(3,146)	(3,461)
Stock Options/Issuances	514	565	622	684	753	828
Other, Investments, Net	(40)	0	0	0	0	0
Net From Non-Operating Trar	(3,244)	(2,961)	(1,978)	(2,176)	(2,393)	(2,633)
Net Change In Cash & Equival	(520)	409	2,692	3,355	3,952	4,687
Year-End Cash & Equivalents	3,758	4,167	6,859	10,215	14,167	18,854

Estimated Annual Balance Sheet ($MM)

	2001	2002E	2003E	2004E	2005E	2006E
Current Assets						
Cash/Equivalents	7,972	4,167	6,859	10,215	14,167	18,854
Accounts Receivable	4,630	5,164	5,719	6,161	6,626	7,100
Inventories	2,992	3,346	3,692	3,961	4,243	4,528
Other Current Assets	2,879	3,197	3,526	3,781	4,048	4,318
Total Current Assets	18,473	15,875	19,796	24,118	29,085	34,799
Property, Plant & Equipment	7,719	8,037	8,265	8,385	8,380	8,228
Intangible Assets	9,077	8,805	8,541	8,284	8,036	7,795
Other	3,219	3,541	3,895	4,284	4,713	5,184
Total Assets	38,488	36,258	40,496	45,072	50,213	56,006
Liabilities & Equity						
Current Liabilities	7,479	8,400	9,369	10,165	11,011	11,883
Short Term Debt	565	2,539	2,539	2,539	2,539	2,539
Total Current liabilities	8,044	10,939	11,908	12,704	13,550	14,422
Long Term Debt	2,217	2,217	2,217	2,217	2,217	2,217
Other Liabilities	3,994	1,393	1,484	1,619	1,801	2,033
Equity	22,204	21,709	24,887	28,532	32,645	37,334
Total Liabilities & Equity	38,488	36,258	40,496	45,072	50,213	56,006

Estimated Select Financial Data ($MM)

	2001	2002E	2003E	2004E	2005E	2006E
Earnings Per Share	$1.91	$2.24	$2.62	$2.97	$3.36	$3.81
% Change	17.0%	17.4%	17.0%	13.6%	12.9%	13.4%
Dividends Per Share	$0.66	$0.78	$0.91	$1.05	$1.21	$1.39
% Change	18.1%	19.3%	16.2%	15.0%	15.0%	15.0%
Payout Ratio	35%	35%	35%	35%	36%	36%
Total Cash (Year End)	7,972	4,167	6,859	10,215	14,167	18,854
Total Debt (Year End)	2,782	4,756	4,756	4,756	4,756	4,756
Net Cash/(Debt)	5,190.0	(588.6)	2,103.3	5,458.6	9,411.0	14,098.3
Per Share	$1.67	($0.19)	$0.70	$1.80	$3.11	$4.66
EBIT	7,885	9,678	11,063	12,428	13,906	15,674
EBITDA	9,490	11,459	13,041	14,623	16,342	18,378
CFFO	7,371	8,621	9,895	11,191	12,595	14,226
Per Share	$2.37	$2.82	$3.27	$3.70	$4.16	$4.70
% Change	12.4%	19.0%	16.0%	13.1%	12.5%	12.9%
FCF	5,086	3,836	4,670	5,531	6,346	7,320
Per Share	$1.63	$1.25	$1.54	$1.83	$2.10	$2.42
% Change	45.0%	-23.3%	23.1%	18.4%	14.7%	15.4%
Total Equity (Year End)	22,204	21,709	24,887	28,532	32,645	37,334
Book Value Per Share	$7.13	$7.10	$8.22	$9.43	$10.79	$12.34
Total Debt/Total Equity	12.5%	21.9%	19.1%	16.7%	14.6%	12.7%
Total Debt/Total Capital	11.1%	18.0%	16.0%	14.3%	12.7%	11.3%
Return on Invested Cap.	23.7%	28.0%	30.8%	30.5%	30.1%	29.9%
Return On Average Equity	27.8%	31.2%	34.0%	33.7%	33.2%	32.9%
Return on Average Assets	15.7%	18.1%	20.5%	20.6%	20.7%	21.0%
Days Receivables (# Days)	52.3	51.9	51.6	51.2	50.8	50.5
% Change	-7.1%	-0.7%	-0.7%	-0.7%	-0.7%	-0.7%
Days Inv. (# of Days)	114.0	116.9	117.0	117.3	117.3	117.2
% Change	-4.2%	2.6%	0.0%	0.3%	0.0%	0.0%
Inventory Turnover	3.20	3.12	3.12	3.11	3.11	3.11
% Change	4.4%	-2.5%	0.0%	-0.3%	0.0%	0.0%
Inventory/Revenue	0.09	0.09	0.09	0.09	0.09	0.09
% Change	-4.9%	-0.4%	-1.1%	-1.1%	-1.1%	-1.1%

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SG COWEN RATING DEFINITIONS

Rating	Definition
Strong Buy (1)	Stock expected to outperform the market by over 25%
Outperform (2)	Stock expected to outperform the market by 10-25%
Market Perform (3)	Stock expected to out/underperform the market by +/-10%
Underperform (4)	Stock expected to underperform the market by at least 10%

Assumptions: Time horizon is 12 months; market is flat over forecast period.

SG COWEN RATING DEFINITIONS PRIOR TO 9/9/2002

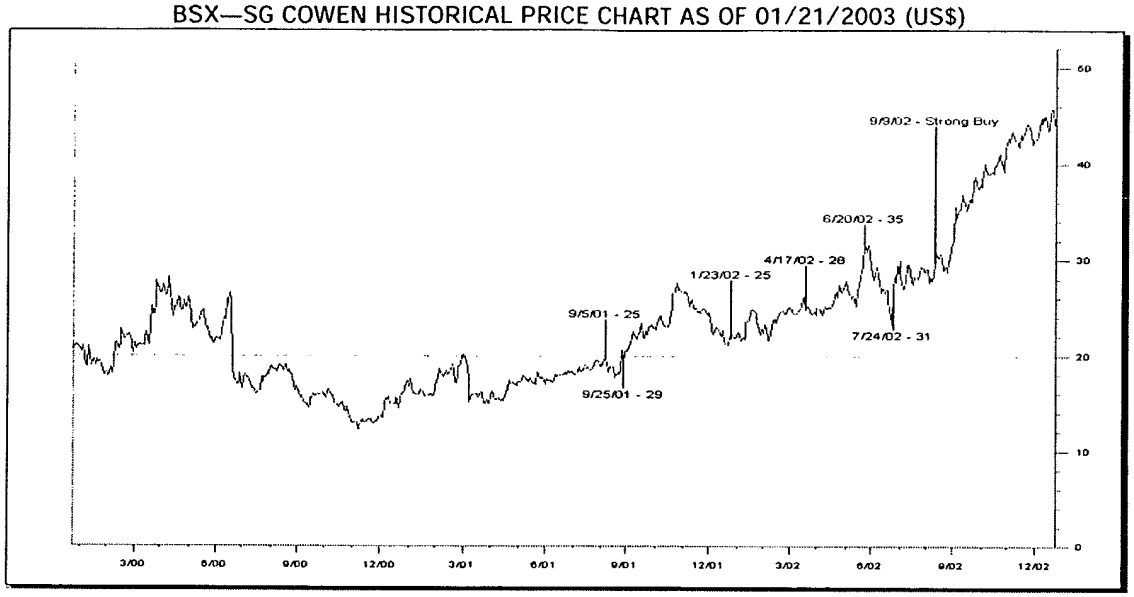
Rating	Definition
Strong Buy (1)	Analyst expects the stock to outperform the market over the next 6-12 months
Buy (2)	Analyst expects the stock to outperform the market over the next 12-18 months
Neutral (3)	Analyst expects the stock to perform in line with the market over the next 12 months
Underperform (4)	Analyst expects the stock to underperform the market over the next 12 months

SG COWEN RATING ALLOCATION (a)

Rating	Pct of companies under coverage with this rating	Pct for which Investment Banking services have been provided within the past 12 months
Buy (b)	54.1%	6.4%
Hold (c)	39.5%	1.4%
Sell (d)	6.4%	0.6%

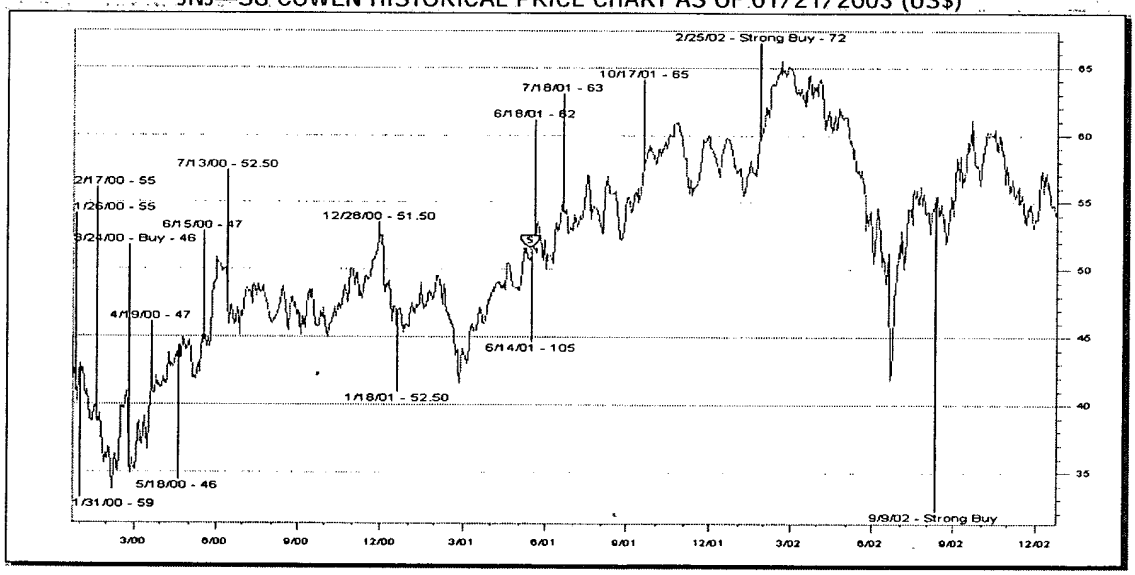
(a) As of 12/31/2002. (b) Includes "Strong Buy" and "Outperform" rated stocks as defined in the SG Cowen's rating system (see above). (c) Corresponds to "Market Perform" as defined in the SG Cowen's ratings system (see above). (d) Corresponds to "Underperform" as defined in the SG Cowen's ratings system (see above).
Note: "Buy," "Hold" and "Sell" are not defined SG Cowen ratings and should not be construed as investment options. Rather, these ratings are used illustratively to comply with NASD and NYSE regulations.

BSX—SG COWEN HISTORICAL PRICE CHART AS OF 01/21/2003 (US$)



Initiated on 01/05/96 with Buy ; Rating as of 04/21/99 was Strong Buy; Price Target as of 06/21/99 was $41.00; Coverage In Transition on 09/28/00; Coverage Resumed on 09/05/01 with Strong Buy and Price Target $25.00

JNJ—SG COWEN HISTORICAL PRICE CHART AS OF 01/21/2003 (US$)



Initiated on 05/01/94 with Neutral ; Rating as of 12/04/98 was Strong Buy; Price Target as of 08/05/99 was $59.00

SG COWEN
Scott Wilkin/Sara Michelmore
(212) 278 4519/4509

Johnson & Johnson
JNJ: $57
Rated 1 (Strong Buy)

January 13, 2003

A Positive Update on JNJ's Remicade and Ortho EVRA Products

	EPS*	P/E	Quarterly EPS			
	FY-Dec		Q1	Q2	Q3	Q4
2001A	$1.91		$0.50	$0.51	$0.50	$0.39
2002E	$2.23	26x	$0.59A	$0.60A	$0.57A	$0.46
2003E	$2.60	23x	$0.69	$0.70	$0.69	$0.53

GAAP EPS excluding one-time non-recurring charges.

Conclusion:
Based on our analysis, recent concerns surrounding growth prospects for J&J's Remicade and Ortho Contraceptive franchise 2003 appear overdone. We remain bullish on Remicade, and look for non-RA indications including Crohn's disease and ulcerative colitis to drive strong sales growth. In addition, Ortho Evra prescriptions have accelerated as of late giving us increased confidence in our estimates for J&J's Ortho Contraceptive franchise.

Key Points:
1. Reiterating above consensus sales 2006 forecast for Remicade ($3.3B) despite increased RA competition.
2. Maintain bullish view on opportunity for maintenance Crohn's which we estimate at over $1B.
3. Remicade in ulcerative colitis now nationally covered by Medicare - another large market opportunity.
4. We remain comfortable with our assumptions for generic impact to the Contraceptive franchise in '03 and '04, particularly given recent strength in Ortho Evra prescriptions trends.
5. Ortho Evra prescriptions suggest run rate exiting '02 in excess of $120MM, ahead of our expectations.

Investment Thesis:
We favor J&J for its accelerating EPS growth (15-16%) and robust top-line growth (9-10%) prospects over the next several years, and believe our estimates have ample opportunity for upside. We view its growth fundamentals as the best in med tech; both the Pharmaceutical and Medical Devices & Diagnostics divisions are poised to deliver double-digit growth for the next several years. Also, J&J's impressive free cash flow gives the company unrivaled financial flexibility. Key franchises in the Pharmaceutical business – including Procrit, Remicade, and Topamax – should support sustained a double-digit growth rate puts that company at the upper end of performance in the drug group. In the Medical Devices and Diagnostics division, numerous drivers, including the Cypher drug-coated stent and Lifescan diabetes products support an increase to double-digit growth over the next several years. Risks to our outlook include approval and launch timing of the Cypher stent, increased competition in the EPO market, and the potential for generic competition to key pharmaceutical franchises.

Discussion:
Potential for Remicade Extends Well Beyond RA In 2003
There has been continued concern that Remicade sales growth will slow in 2003 from increased competition in the RA market from Humira and the re-launch of Enbrel. We believe our assumptions for Remicade in RA are reasonable in the face of increased competition, and look for new opportunities beyond RA - particularly Crohn's maintenance – to sustain solid growth for the product going forward well ahead of

consensus views. We note our RA assumptions assume the drug captures only 20% of new RA patients going forward which we believe is reasonable given that over 1/3 of the patient population is covered under Medicare. We believe expansion of Remicade's Crohn's label to include a maintenance schedule represents a market opportunity for the drug in excess of $1B based on an increase in average yearly dosing coupled with expansion in drug penetration as suggested by our proprietary survey work.

REMICADE SALES BUILDUP BY INDICATION ($MM)

	2000	2001E	2002E	2003E	2004E	2005E	2006E	01-06E CAGR
TOTAL	**$371**	**$721**	**$1,231**	**$1,690**	**$2,253**	**$2,836**	**$3,307**	**36%**
US	305	687	1,132	1,561	2,088	2,637	3,072	35%
Crohn's Dis./Other GI	120	250	358	588	850	1,083	1,201	37%
Rheumatoid Arthritis	186	436	775	974	1,134	1,293	1,423	27%
Psoriasis	0	0	0	0	52	106	200	-
Psoriatic Arthritis	0	0	0	0	52	100	175	-
Ankylosing Spon./Other	0	0	0	0	0	51	75	-
International	66	34	99	129	165	198	234	47%

Source: Company reports and SG Cowen research.

In addition to Crohn's, we see solid opportunities for the drug in 2003 in expanded indications for maintenance fistulizing Crohn's (currently under priority review), as well as ulcerative colitis (Phase III) and psoriatic arthritis (Phase III). Remicade is already used off-label for maintenance fistulizing disease but we expect expanded labeling will aid in driving increased average dosing for Crohn's disease in line with our current expectations.

Growing coverage of the drug for other indications off label could drive some upside to our model in 2003. Namely, use of Remicade for ulcerative colitis – a similar patient opportunity to Crohn's – is now nationally covered by CMS as of August 2002 for refractory patients. Although the number of patients with ulcerative colitis covered by Medicare is a modest percentage of the total, we believe it sets a precedent for private payors. Due to the favorable economics, we believe it is likely that the private pay sector will follow suit – refractory patients progress to colectomy surgery (6,000+/year) at a cost in excess of $25K per patient. Coverage for Remicade for the treatment of psoriatic arthritis has also made progress, with 27/47 regional carriers covering use of the drug and some private pay coverage. Although a significantly smaller opportunity, the drug is also being covered on a regional basis for ankylosing spondylytis and has coverage by some private payors such as Aetna.

Ortho Evra Prescriptions Have Been Very Strong, Giving Us Confidence In Contraceptive Estimates

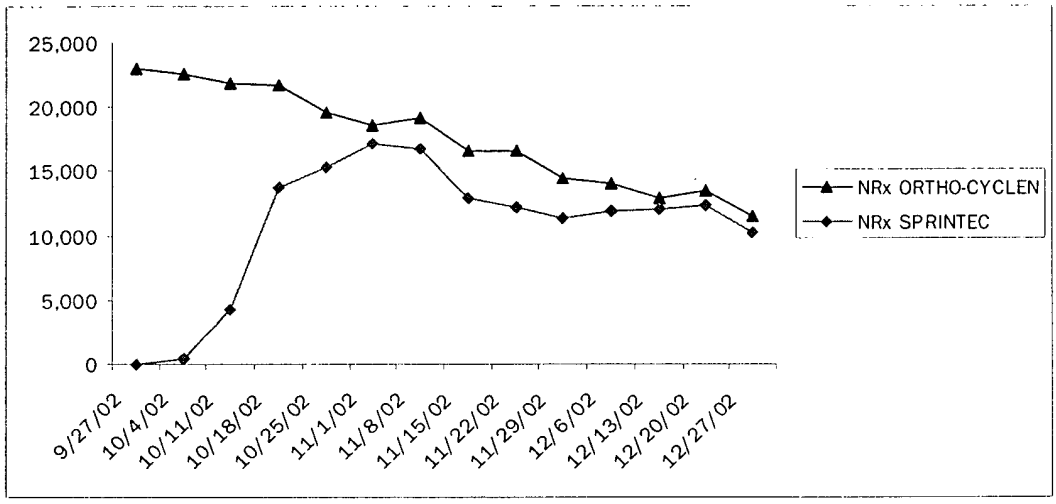
In November, we made a downward adjustment to our forecast for oral contraceptives to reflect generic competition to three Ortho contraceptive products – Ortho Cyclen, Ortho Novum 7/7/7 and Ortho Tri Cyclen. The first generic to Ortho Cyclen was launched in September and a second is on the way, 2 generics to Ortho Novum 7/7/7 were launched last week. We anticipate generic competition to Ortho Tri Cyclen in Q1:04; although it could come as early as September 2003.

	2001	2002E	2003E	2004E	2005E	2006E
Ortho Contraceptives	892	934	769	550	583	653
Y/Y (%)	6%	5%	-18%	-29%	6%	12%

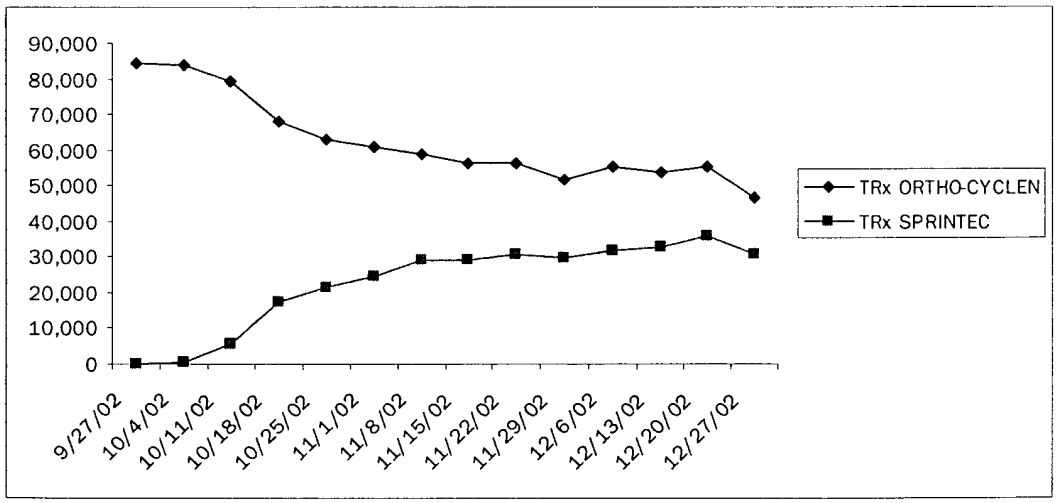
We have continued to monitor the prescriptions of Ortho Cyclen, as well as other major contraceptive brands, which have faced generics in the last 12-18 months including Wyeth's Alesse. In addition we have tracked the progress of both Ortho Evra and Ortho Tri Cyclen Lo - recent J&J launches that are expected to

partially offset declines to the business due to generics, although we do not assume these drugs will prevent a decline in the franchise. To date, we remain comfortable with our underlying assumptions regarding generic competition to oral contraceptives. After an initial drop of 30-35% of total prescriptions Ortho Cyclen substitution have somewhat stabilized. New prescriptions have continued to drop, but in line with our expectations for 2003. Recent examples, such as Wyeth's Alesse, support our view that generic substitution is somewhat slower than a normal generic adoption curve and at 12 months the branded product retains roughly 50% of total and new prescriptions, and 40% of total and new prescriptions at 18 months.

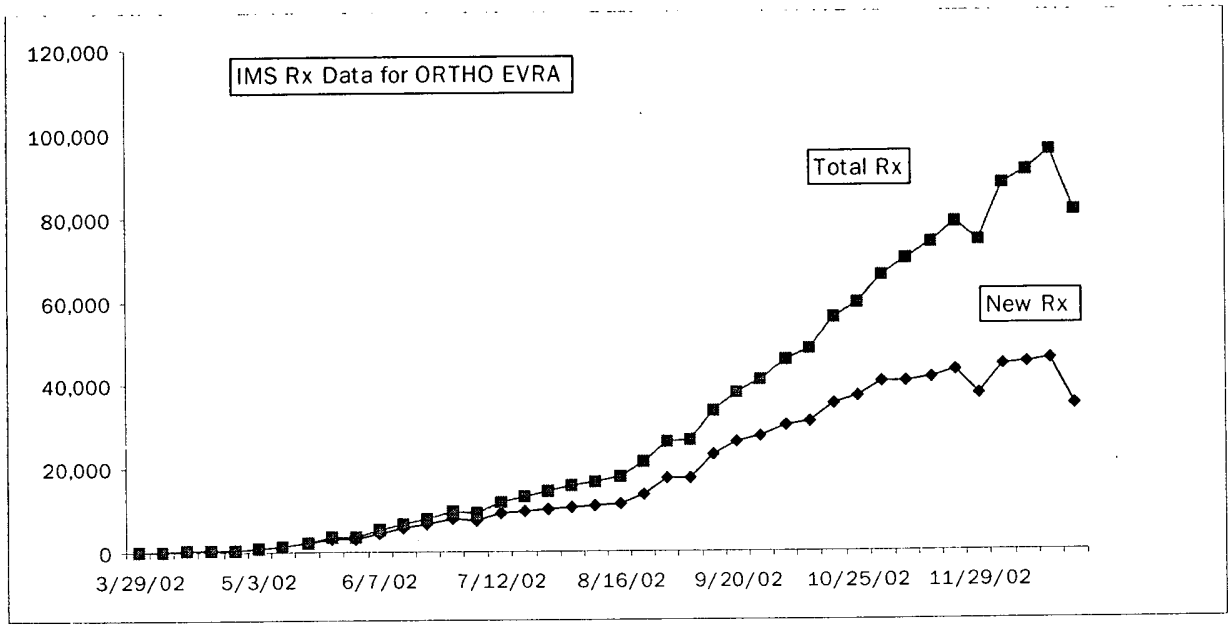
IMS RX DATA FOR ORTHO CYCLEN – NEW PRESCRIPTIONS



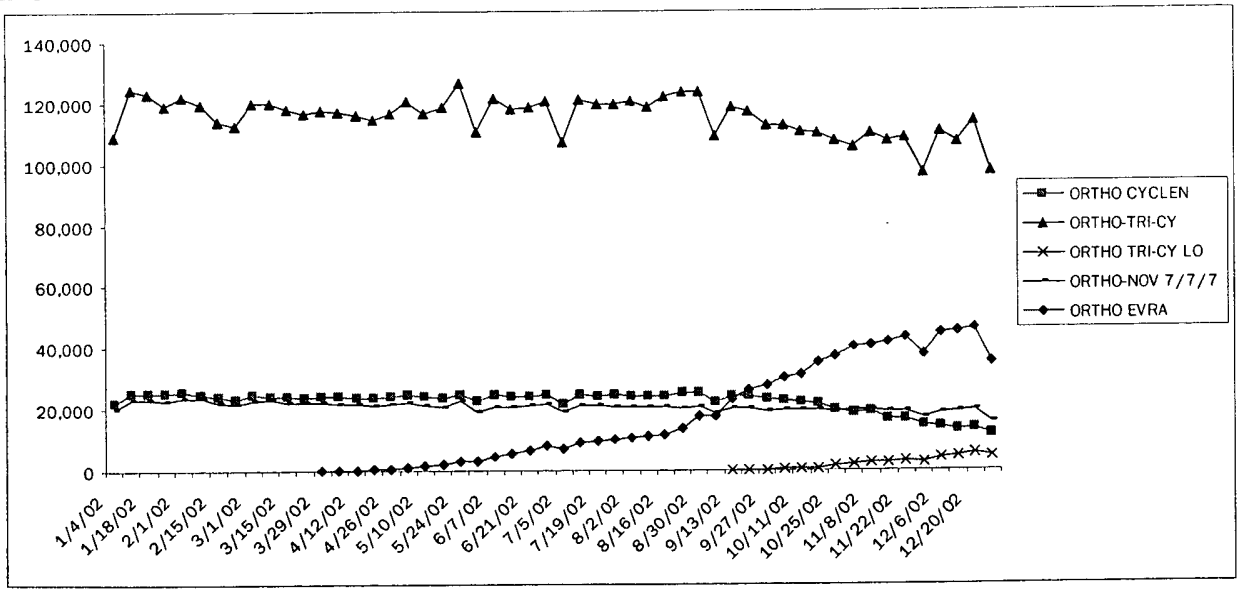
IMS RX DATA FOR ORTHO CYCLEN – TOTAL PRESCRIPTIONS



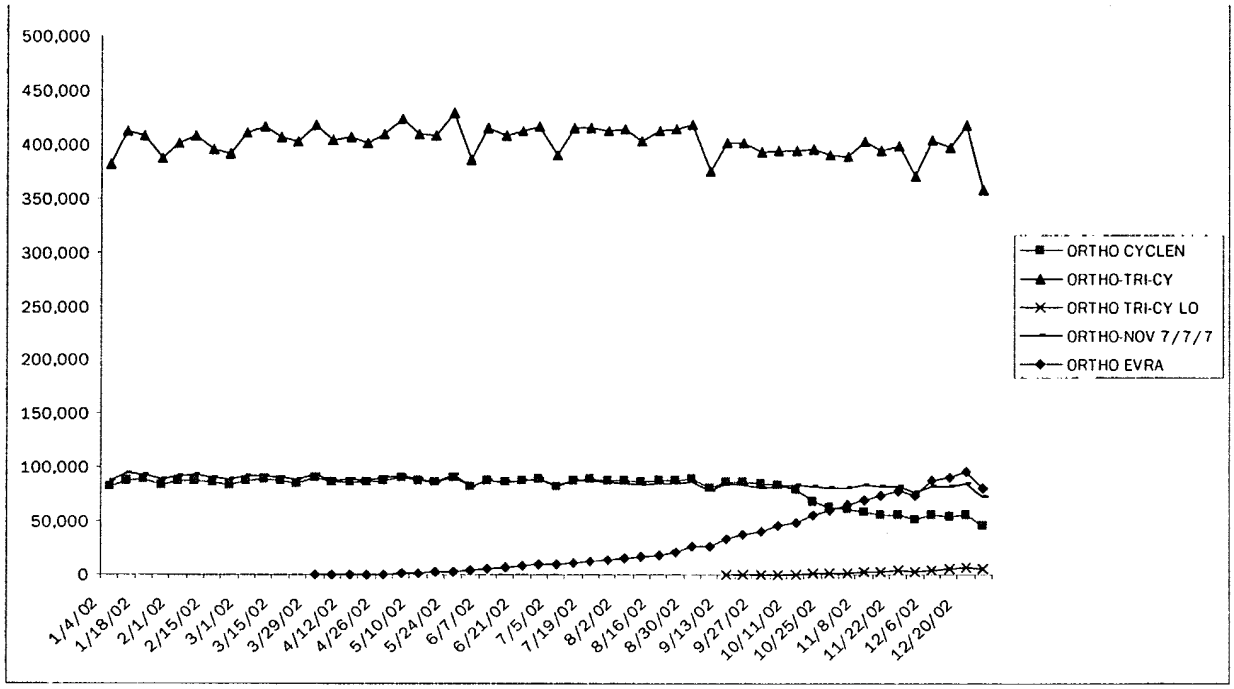
On a positive note, prescription growth for Ortho Evra has accelerated significantly since August – with total prescriptions growing from 15,000 to close to 100,000 exiting 2002, and new prescription growth remaining robust. The current Rx run rate exiting 2002 is over $120MM in sales which is ahead of our expectations and puts Evra in a solid number 2 spot with the Ortho Contraceptives franchise heading into 2003.



IMS RX DATA – NEW RX OF KEY ORTHO CONTRACEPTIVE FRANCHISES



IMS RX DATA – TOTAL RX OF KEY ORTHO CONTRACEPTIVE FRANCHISES



	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1A	Q2 A	Q3 A	Q4 E	Year	Q1E	Q2 E	Q3 E	Q4 E	Year
Worldwide															
Med. Device & Diag.	2,735	2,785	2,772	2,854	11,146	2,958	3,166	3,141	3,158	12,423	3,249	3,702	3,729	3,758	14,438
Pharmaceutical	3,490	3,864	3,677	3,820	14,851	4,180	4,258	4,277	4,443	17,159	4,568	4,634	4,791	4,966	18,959
Base J&J	3,260	3,609	3,398	3,563	13,830	3,895	3,974	3,931	4,107	15,907	4,218	4,296	4,368	4,547	17,429
Alza	230	255	280	257	1,022	286	284	346	336	1,252	350	338	424	419	1,531
Consumer	1,630	1,530	1,609	1,551	6,320	1,604	1,649	1,661	1,599	6,513	1,651	1,694	1,704	1,643	6,692
WW Total	**7,855**	**8,179**	**8,058**	**8,225**	**32,317**	**8,743**	**9,073**	**9,079**	**9,200**	**36,095**	**9,468**	**10,030**	**10,225**	**10,367**	**40,090**
U.S.															
Med. Device & Diag.	1,463	1,530	1,569	1,574	6,136	1,663	1,758	1,740	1,738	6,899	1,846	2,174	2,226	2,236	8,482
Pharmaceutical	2,356	2,722	2,511	2,651	10,240	2,958	2,934	2,939	3,155	11,986	3,225	3,172	3,308	3,543	13,248
Base J&J	2,126	2,467	2,232	2,394	9,219	2,672	2,650	2,593	2,818	10,733	2,875	2,834	2,884	3,123	11,717
Alza	230	255	280	257	1,022	286	284	346	336	1,252	350	338	424	419	1,531
Consumer	896	807	896	850	3,449	900	907	910	883	3,600	929	935	936	906	3,706
U.S. Total	**4,715**	**5,059**	**4,976**	**5,075**	**19,825**	**5,521**	**5,599**	**5,589**	**5,776**	**22,485**	**6,000**	**6,281**	**6,470**	**6,685**	**25,437**
International															
Med. Device & Diag.	1,272	1,255	1,203	1,280	5,010	1,295	1,408	1,401	1,419	5,523	1,403	1,527	1,503	1,522	5,956
Pharmaceutical	1,134	1,142	1,166	1,169	4,611	1,223	1,324	1,338	1,289	5,173	1,343	1,462	1,484	1,423	5,711
Consumer	735	723	713	701	2,872	704	742	751	716	2,913	722	760	768	736	2,986
Int. Total	**3,141**	**3,120**	**3,082**	**3,150**	**12,493**	**3,221**	**3,474**	**3,490**	**3,424**	**13,610**	**3,468**	**3,749**	**3,755**	**3,682**	**14,653**
Growth Analysis															
Worldwide															
Med. Device & Diag.						8.2%	13.7%	13.3%	10.6%	11.5%	9.8%	16.9%	18.7%	19.0%	16.2%
Pharmaceutical						19.8%	10.2%	16.3%	16.3%	15.5%	9.3%	8.8%	12.0%	11.8%	10.5%
Base J&J						19.5%	10.1%	15.7%	15.3%	15.0%	8.3%	8.1%	11.1%	10.7%	9.6%
Alza						24.2%	11.5%	23.8%	30.9%	22.6%	22.3%	18.9%	22.5%	24.7%	22.2%
Consumer						-1.6%	7.8%	3.2%	3.1%	3.1%	2.9%	2.7%	2.6%	2.7%	2.8%
WW Total						**11.3%**	**10.9%**	**12.7%**	**11.9%**	**11.7%**	**8.3%**	**10.5%**	**12.6%**	**12.7%**	**11.1%**
U.S.															
Med. Device & Diag.						13.7%	14.9%	10.9%	10.4%	12.4%	11.0%	23.7%	27.9%	28.6%	22.9%
Pharmaceutical						25.6%	7.8%	17.0%	19.0%	17.0%	9.0%	8.1%	12.6%	12.3%	10.5%
Base J&J						25.7%	7.4%	16.2%	17.7%	16.4%	7.6%	7.0%	11.2%	10.8%	9.2%
Alza						24.2%	11.5%	23.8%	30.9%	22.6%	22.3%	18.9%	22.5%	24.7%	22.2%
Consumer						0.5%	12.4%	1.6%	3.9%	4.4%	3.2%	3.1%	2.9%	2.7%	3.0%
U.S. Total						**17.1%**	**10.7%**	**12.3%**	**13.8%**	**13.4%**	**8.7%**	**12.2%**	**15.8%**	**15.7%**	**13.1%**
International															
Med. Device & Diag.						1.8%	12.2%	16.5%	10.9%	10.2%	8.3%	8.5%	7.3%	7.3%	7.8%
Pharmaceutical						7.8%	15.9%	14.8%	10.3%	12.2%	9.8%	10.4%	10.9%	10.4%	10.4%
Consumer						-4.2%	2.6%	5.3%	2.2%	1.4%	2.6%	2.4%	2.2%	2.8%	2.5%
Int. Total						**2.6%**	**11.3%**	**13.2%**	**8.7%**	**8.9%**	**7.6%**	**7.9%**	**7.6%**	**7.5%**	**7.7%**

Source: Company reports and SG Cowen estimates.

	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	**Q2 A**	**Q3 A**	**Q4 A**	**Year**	**Q1A**	**Q2 A**	**Q3 A**	**Q4 E**	**Year**	**Q1E**	**Q2 E**	**Q3 E**	**Q4 E**	**Year**
Total Revenue	**7,855**	**8,179**	**8,058**	**8,225**	**32,317**	**8,743**	**9,073**	**9,079**	**9,200**	**36,095**	**9,468**	**10,030**	**10,225**	**10,367**	**40,090**
Cost of Goods Sold	2,311	2,372	2,396	2,502	9,581	2,457	2,582	2,611	2,753	10,403	2,651	2,824	2,930	3,050	11,456
Gross Profit	**5,544**	**5,807**	**5,662**	**5,723**	**22,736**	**6,286**	**6,491**	**6,468**	**6,447**	**25,692**	**6,816**	**7,206**	**7,295**	**7,317**	**28,634**
R&D	759	829	899	1,104	3,591	831	932	952	1,272	3,987	947	1,045	1,099	1,458	4,549
SG&A	2,666	2,802	2,703	3,089	11,260	2,843	3,017	3,006	3,313	12,179	3,022	3,265	3,365	3,639	13,291
Operating Income	**2,119**	**2,176**	**2,060**	**1,530**	**7,885**	**2,612**	**2,542**	**2,510**	**1,863**	**9,527**	**2,847**	**2,895**	**2,831**	**2,220**	**10,793**
Interest	(92)	(70)	(67)	(74)	(303)	(42)	(30)	(12)	(20)	(104)	(28)	(40)	(55)	(65)	(188)
Other	(6)	15	(18)	55	46	33	(45)	129	(20)	97	(15)	(15)	(15)	(15)	(60)
Pretax Income	**2,217**	**2,231**	**2,145**	**1,549**	**8,142**	**2,621**	**2,617**	**2,393**	**1,903**	**9,534**	**2,890**	**2,950**	**2,901**	**2,300**	**11,041**
Taxes	665	647	579	339	2,230	787	774	668	476	2,705	824	841	827	655	3,147
Net Income	**1,552**	**1,584**	**1,566**	**1,210**	**5,912**	**1,834**	**1,843**	**1,725**	**1,427**	**6,829**	**2,066**	**2,110**	**2,074**	**1,644**	**7,894**
Net Interest Add Back*	9	9	0	0	18	0	0	0	0	0	0	0	0	0	0
Adj. Net Income	**1,561**	**1,593**	**1,566**	**1,210**	**5,930**	**1,834**	**1,843**	**1,725**	**1,427**	**6,829**	**2,066**	**2,110**	**2,074**	**1,644**	**7,894**
Shares (MM)	3,107	3,111	3,111	3,121	3,112	3,115	3,069	3,027	3,027	3,060	3,030	3,031	3,033	3,035	3,032
Earnings Per Share	**$0.50**	**$0.51**	**$0.50**	**$0.39**	**$1.91**	**$0.59**	**$0.60**	**$0.57**	**$0.47**	**$2.23**	**$0.68**	**$0.70**	**$0.68**	**$0.54**	**$2.60**
Margin Analysis															
Gross Profit	70.6%	71.0%	70.3%	69.6%	70.4%	71.9%	71.5%	71.2%	70.1%	71.2%	72.0%	71.8%	71.3%	70.6%	71.4%
R&D	9.7%	10.1%	11.2%	13.4%	11.1%	9.5%	10.3%	10.5%	13.8%	11.0%	10.0%	10.4%	10.7%	14.1%	11.3%
SG&A	33.9%	34.3%	33.5%	37.6%	34.8%	32.5%	33.3%	33.1%	36.0%	33.7%	31.9%	32.6%	32.9%	35.1%	33.2%
Operating Income	27.0%	26.6%	25.6%	18.6%	24.4%	29.9%	28.0%	27.6%	20.3%	26.4%	30.1%	28.9%	27.7%	21.4%	26.9%
Pretax Income	28.2%	27.3%	26.6%	18.8%	25.2%	30.0%	28.8%	26.4%	20.7%	26.4%	30.5%	29.4%	28.4%	22.2%	27.5%
Net Income	19.8%	19.4%	19.4%	14.7%	18.3%	21.0%	20.3%	19.0%	15.5%	18.9%	21.8%	21.0%	20.3%	15.9%	19.7%
Tax Rate	30.0%	29.0%	27.0%	21.9%	27.4%	30.0%	29.6%	27.9%	25.0%	28.4%	28.5%	28.5%	28.5%	28.5%	28.5%
Growth Analysis															
Total Revenue						11.3%	10.9%	12.7%	11.9%	11.7%	8.3%	10.5%	12.6%	12.7%	11.1%
Gross Profit						13.4%	11.8%	14.2%	12.7%	13.0%	8.4%	11.0%	12.8%	13.5%	11.5%
R&D	12.1%	16.3%	21.7%	13.1%	15.7%	9.5%	12.4%	5.9%	15.2%	11.0%	14.0%	12.2%	15.4%	14.6%	14.1%
SG&A						6.6%	7.7%	11.2%	7.2%	8.2%	6.3%	8.2%	11.9%	9.9%	9.1%
Operating Income	15.0%	16.6%	18.3%	31.3%	19.2%	23.2%	16.8%	21.8%	21.8%	20.8%	9.0%	13.9%	12.8%	19.1%	13.3%
Pretax Income	15.8%	16.6%	17.0%	24.9%	18.0%	18.2%	17.3%	11.6%	22.9%	17.1%	10.3%	12.7%	21.2%	20.8%	15.8%
Net Income	16.6%	16.2%	18.3%	23.3%	18.3%	18.1%	16.4%	10.2%	18.0%	15.5%	12.7%	14.5%	20.2%	15.2%	15.6%
EPS	14.2%	15.4%	17.6%	22.0%	17.0%	17.1%	17.3%	13.2%	21.6%	17.1%	15.9%	15.9%	20.0%	14.9%	16.6%

Source: Company reports and SG Cowen estimates.

Estimated Annual Revenue ($MM)

	FY2000	2001	2002E	2003E	2004E	2005E	2006E	01-06E Annual Growth Rate	% 00 - 01	% 01 - 02	% 02 - 03	% 03 - 04	% 04 - 05	% 05 - 06
Total Divisional Summary	$29,847	$32,417	$36,095	$40,090	$43,306	$47,111	$50,817	9%	9%	11%	11%	8%	9%	8%
Domestic	17,708	19,869	22,486	25,437	27,455	30,083	32,578	10%	12%	13%	13%	8%	10%	8%
International	12,139	12,548	13,609	14,653	15,851	17,028	18,239	8%	3%	8%	8%	8%	7%	7%
Med Device & Diag.	**10,281**	**11,211**	**12,423**	**14,438**	**15,500**	**16,907**	**18,103**	10%	9%	11%	16%	7%	9%	7%
Domestic	5,506	6,181	6,900	8,482	9,028	9,944	10,671	12%	12%	12%	23%	6%	10%	7%
International	4,775	5,030	5,523	5,956	6,472	6,964	7,432	8%	5%	10%	8%	9%	8%	7%
Pharmaceutical	**12,662**	**14,851**	**17,159**	**18,960**	**20,866**	**22,998**	**25,249**	11%	17%	16%	10%	10%	10%	10%
Domestic	8,442	10,240	11,986	13,248	14,561	16,102	17,708	12%	21%	17%	11%	10%	11%	10%
International	4,220	4,611	5,173	5,711	6,306	6,896	7,542	10%	9%	12%	10%	10%	9%	9%
Consumer	**6,904**	**6,356**	**6,513**	**6,692**	**6,939**	**7,206**	**7,464**	3%	-8%	2%	3%	4%	4%	4%
Domestic	3,760	3,449	3,600	3,706	3,866	4,037	4,199	4%	-8%	4%	3%	4%	4%	4%
International	3,144	2,907	2,913	2,986	3,073	3,168	3,265	2%	-8%	0%	3%	3%	3%	3%

Johnson & Johnson Estimated Annual Profit and Loss Statement ($MM)

	FY2000	2001	2002E	2003E	2004E	2005E	2006E	01-06E Annual Growth Rate
Total Revenues	**29,846**	**32,317**	**36,095**	**40,090**	**43,306**	**47,111**	**50,817**	**9.5%**
Cost of Goods Sold	8,908	9,581	10,403	11,456	12,202	13,132	13,962	
Gross Profit	**20,938**	**22,736**	**25,692**	**28,634**	**31,104**	**33,979**	**36,855**	**10.1%**
R&D	3,105	3,591	3,987	4,549	5,022	5,582	5,970	
SG&A	11,218	11,260	12,179	13,291	14,003	14,818	15,527	
Operating Income	**6,615**	**7,885**	**9,527**	**10,793**	**12,079**	**13,579**	**15,358**	**14.3%**
Interest, Net	(225)	(303)	(104)	(188)	(385)	(555)	(630)	
Other, Net	(61)	46	97	(60)	(60)	(60)	(60)	
Pretax Income	**6,901**	**8,142**	**9,534**	**11,041**	**12,524**	**14,194**	**16,048**	**14.5%**
Taxes	1,903	2,230	2,705	3,147	3,569	4,045	4,574	
Net Income	**4,998**	**5,912**	**6,829**	**7,894**	**8,955**	**10,148**	**11,474**	**14.2%**
Net Int. Add Back	**45**	**18**	0	0	0	0	0	
Adj. Net Income	**5,043**	**5,930**	**6,829**	**7,894**	**8,955**	**10,148**	**11,474**	**14.1%**
Shares (MM)	3,097	3,112	3,060	3,032	3,032	3,032	3,032	
Earnings Per Share	**$1.63**	**$1.91**	**$2.23**	**$2.60**	**$2.95**	**$3.35**	**$3.78**	**14.7%**
Growth Analysis								
Total Revenues	6.6%	8.3%	11.7%	11.1%	8.0%	8.8%	7.9%	
Gross Profit	7.3%	8.6%	13.0%	11.5%	8.6%	9.2%	8.5%	
R&D	12.2%	15.7%	11.0%	14.1%	10.4%	11.1%	7.0%	
SG&A	4.3%	0.4%	8.2%	9.1%	5.4%	5.8%	4.8%	
Operating Income	10.5%	19.2%	20.8%	13.3%	11.9%	12.4%	13.1%	
Pretax Income	17.0%	18.0%	17.1%	15.8%	13.4%	13.3%	13.1%	
Net Income	17.7%	18.3%	15.5%	15.6%	13.4%	13.3%	13.1%	
Earnings Per Share	18.9%	17.0%	17.1%	16.6%	13.4%	13.3%	13.1%	
Margin Analysis								
Gross Margin	70.2%	70.4%	71.2%	71.4%	71.8%	72.1%	72.5%	
R&D	10.4%	11.1%	11.0%	11.3%	11.6%	11.8%	11.7%	
SG&A	37.6%	34.8%	33.7%	33.2%	32.3%	31.5%	30.6%	
Operating Income	22.2%	24.4%	26.4%	26.9%	27.9%	28.8%	30.2%	
Pretax Income	23.1%	25.2%	26.4%	27.5%	28.9%	30.1%	31.6%	
Net Income	16.7%	18.3%	18.9%	19.7%	20.7%	21.5%	22.6%	
Tax Rate	27.6%	27.4%	28.4%	28.5%	28.5%	28.5%	28.5%	

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SG COWEN RATING DEFINITIONS

Rating	Definition
Strong Buy (1)	Stock expected to outperform the market by over 25%
Outperform (2)	Stock expected to outperform the market by 10-25%
Market Perform (3)	Stock expected to out/underperform the market by +/-10%
Underperform (4)	Stock expected to underperform the market by at least 10%

Assumptions: Time horizon is 12 months; market is flat over forecast period.

SG COWEN RATING DEFINITIONS PRIOR TO 9/9/2002

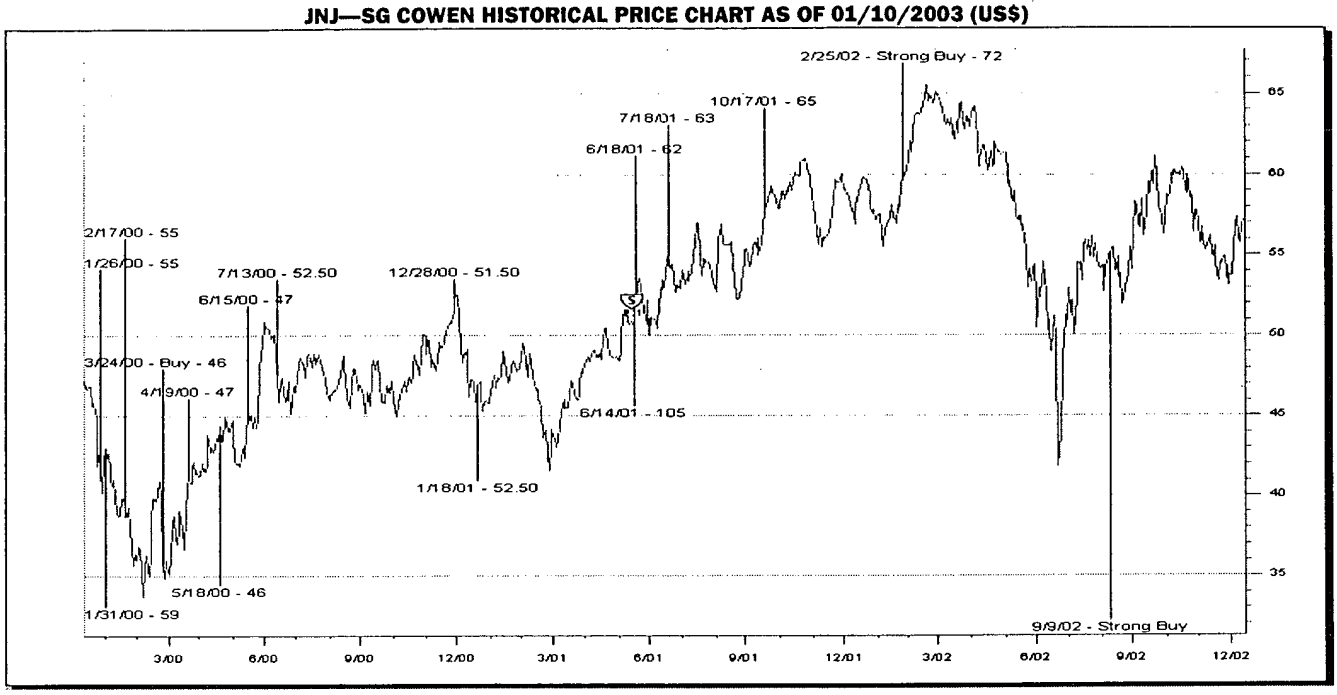
Rating	Definition
Strong Buy (1)	Analyst expects the stock to outperform the market over the next 6-12 months
Buy (2)	Analyst expects the stock to outperform the market over the next 12-18 months
Neutral (3)	Analyst expects the stock to perform in line with the market over the next 12 months
Underperform (4)	Analyst expects the stock to underperform the market over the next 12 months

SG COWEN RATING ALLOCATION (a)

Rating	Pct of companies under coverage with this rating	Pct for which Investment Banking services have been provided within the past 12 months
Buy (b)	54.1%	6.6%
Hold (c)	39.5%	1.4%
Sell (d)	6.4%	0.6%

(a) As of 12/31/2002. (b) Includes "Strong Buy" and "Outperform" rated stocks as defined in the SG Cowen's rating system (see above). (c) Corresponds to "Market Perform" as defined in the SG Cowen's ratings system (see above). (d) Corresponds to "Underperform" as defined in the SG Cowen's ratings system (see above).
Note: "Buy," "Hold" and "Sell" are not defined SG Cowen ratings and should not be construed as investment options. Rather, these ratings are used illustratively to comply with NASD and NYSE regulations.

JNJ—SG COWEN HISTORICAL PRICE CHART AS OF 01/10/2003 (US$)



Initiated on 05/01/94 with Neutral ; Rating as of 12/04/98 was Strong Buy; Price Target as of 08/05/99 was $59.00

SG COWEN
Scott Wilkin/Sara Michelmore
(212) 278 4519/4509

Johnson & Johnson
JNJ: $57
Rated 1 (Strong Buy)

January 13, 2003

A Positive Update on JNJ's Remicade and Ortho EVRA Products

	EPS*	P/E	Quarterly EPS			
	FY-Dec		Q1	Q2	Q3	Q4
2001A	$1.91		$0.50	$0.51	$0.50	$0.39
2002E	$2.23	26x	$0.59A	$0.60A	$0.57A	$0.46
2003E	$2.60	23x	$0.69	$0.70	$0.69	$0.53

GAAP EPS excluding one-time non-recurring charges.

Conclusion:
Based on our analysis, recent concerns surrounding growth prospects for J&J's Remicade and Ortho Contraceptive franchise 2003 appear overdone. We remain bullish on Remicade, and look for non-RA indications including Crohn's disease and ulcerative colitis to drive strong sales growth. In addition, Ortho Evra prescriptions have accelerated as of late giving us increased confidence in our estimates for J&J's Ortho Contraceptive franchise.

Key Points:
1. Reiterating above consensus sales 2006 forecast for Remicade ($3.3B) despite increased RA competition.
2. Maintain bullish view on opportunity for maintenance Crohn's which we estimate at over $1B.
3. Remicade in ulcerative colitis now nationally covered by Medicare - another large market opportunity.
4. We remain comfortable with our assumptions for generic impact to the Contraceptive franchise in '03 and '04, particularly given recent strength in Ortho Evra prescriptions trends.
5. Ortho Evra prescriptions suggest run rate exiting '02 in excess of $120MM, ahead of our expectations.

Investment Thesis:
We favor J&J for its accelerating EPS growth (15-16%) and robust top-line growth (9-10%) prospects over the next several years, and believe our estimates have ample opportunity for upside. We view its growth fundamentals as the best in med tech; both the Pharmaceutical and Medical Devices & Diagnostics divisions are poised to deliver double-digit growth for the next several years. Also, J&J's impressive free cash flow gives the company unrivaled financial flexibility. Key franchises in the Pharmaceutical business – including Procrit, Remicade, and Topamax – should support sustained a double-digit growth rate puts that company at the upper end of performance in the drug group. In the Medical Devices and Diagnostics division, numerous drivers, including the Cypher drug-coated stent and Lifescan diabetes products support an increase to double-digit growth over the next several years. Risks to our outlook include approval and launch timing of the Cypher stent, increased competition in the EPO market, and the potential for generic competition to key pharmaceutical franchises.

Discussion:
Potential for Remicade Extends Well Beyond RA In 2003
There has been continued concern that Remicade sales growth will slow in 2003 from increased competition in the RA market from Humira and the re-launch of Enbrel. We believe our assumptions for Remicade in RA are reasonable in the face of increased competition, and look for new opportunities beyond RA - particularly Crohn's maintenance – to sustain solid growth for the product going forward well ahead of

consensus views. We note our RA assumptions assume the drug captures only 20% of new RA patients going forward which we believe is reasonable given that over 1/3 of the patient population is covered under Medicare. We believe expansion of Remicade's Crohn's label to include a maintenance schedule represents a market opportunity for the drug in excess of $1B based on an increase in average yearly dosing coupled with expansion in drug penetration as suggested by our proprietary survey work.

REMICADE SALES BUILDUP BY INDICATION ($MM)

	2000	2001E	2002E	2003E	2004E	2005E	2006E	01-06E CAGR
TOTAL	**$371**	**$721**	**$1,231**	**$1,690**	**$2,253**	**$2,836**	**$3,307**	**36%**
US	305	687	1,132	1,561	2,088	2,637	3,072	35%
Crohn's Dis./Other GI	120	250	358	588	850	1,083	1,201	37%
Rheumatoid Arthritis	186	436	775	974	1,134	1,293	1,423	27%
Psoriasis	0	0	0	0	52	106	200	-
Psoriatic Arthritis	0	0	0	0	52	100	175	-
Ankylosing Spon./Other	0	0	0	0	0	51	75	-
International	66	34	99	129	165	198	234	47%

Source: Company reports and SG Cowen research.

In addition to Crohn's, we see solid opportunities for the drug in 2003 in expanded indications for maintenance fistulizing Crohn's (currently under priority review), as well as ulcerative colitis (Phase III) and psoriatic arthritis (Phase III). Remicade is already used off-label for maintenance fistulizing disease but we expect expanded labeling will aid in driving increased average dosing for Crohn's disease in line with our current expectations.

Growing coverage of the drug for other indications off label could drive some upside to our model in 2003. Namely, use of Remicade for ulcerative colitis – a similar patient opportunity to Crohn's – is now nationally covered by CMS as of August 2002 for refractory patients. Although the number of patients with ulcerative colitis covered by Medicare is a modest percentage of the total, we believe it sets a precedent for private payors. Due to the favorable economics, we believe it is likely that the private pay sector will follow suit – refractory patients progress to colectomy surgery (6,000+/year) at a cost in excess of $25K per patient. Coverage for Remicade for the treatment of psoriatic arthritis has also made progress, with 27/47 regional carriers covering use of the drug and some private pay coverage. Although a significantly smaller opportunity, the drug is also being covered on a regional basis for ankylosing spondylytis and has coverage by some private payors such as Aetna.

Ortho Evra Prescriptions Have Been Very Strong, Giving Us Confidence In Contraceptive Estimates

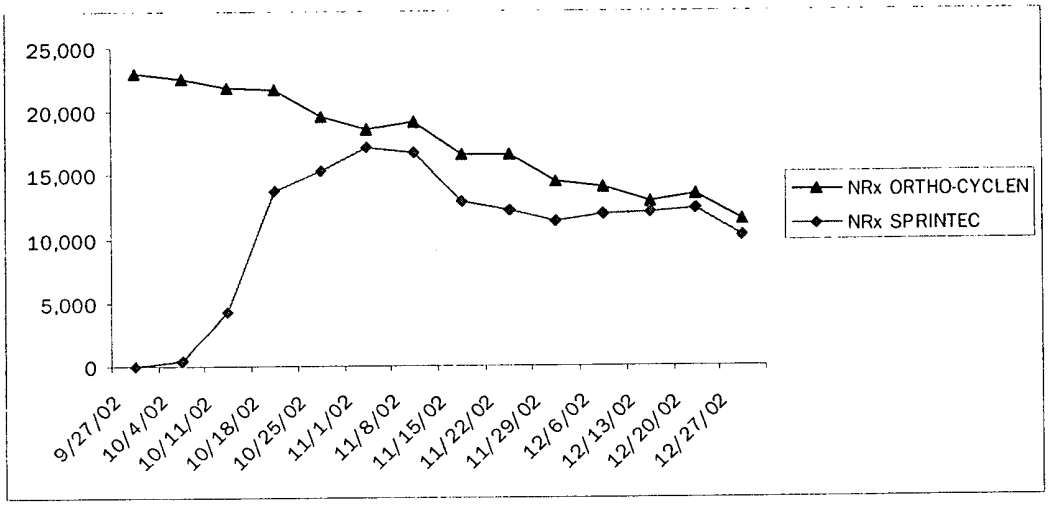
In November, we made a downward adjustment to our forecast for oral contraceptives to reflect generic competition to three Ortho contraceptive products – Ortho Cyclen, Ortho Novum 7/7/7 and Ortho Tri Cyclen. The first generic to Ortho Cyclen was launched in September and a second is on the way, 2 generics to Ortho Novum 7/7/7 were launched last week. We anticipate generic competition to Ortho Tri Cyclen in Q1:04; although it could come as early as September 2003.

	2001	2002E	2003E	2004E	2005E	2006E
Ortho Contraceptives	892	934	769	550	583	653
Y/Y (%)	6%	5%	-18%	-29%	6%	12%

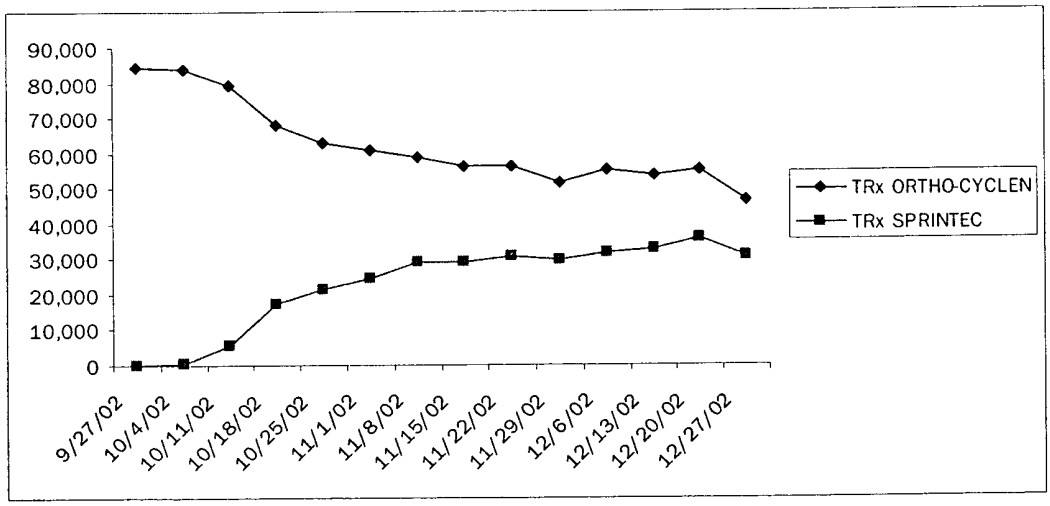
We have continued to monitor the prescriptions of Ortho Cyclen, as well as other major contraceptive brands, which have faced generics in the last 12-18 months including Wyeth's Alesse. In addition we have tracked the progress of both Ortho Evra and Ortho Tri Cyclen Lo - recent J&J launches that are expected to

partially offset declines to the business due to generics, although we do not assume these drugs will prevent a decline in the franchise. To date, we remain comfortable with our underlying assumptions regarding generic competition to oral contraceptives. After an initial drop of 30-35% of total prescriptions Ortho Cyclen substitution have somewhat stabilized. New prescriptions have continued to drop, but in line with our expectations for 2003. Recent examples, such as Wyeth's Alesse, support our view that generic substitution is somewhat slower than a normal generic adoption curve and at 12 months the branded product retains roughly 50% of total and new prescriptions, and 40% of total and new prescriptions at 18 months.

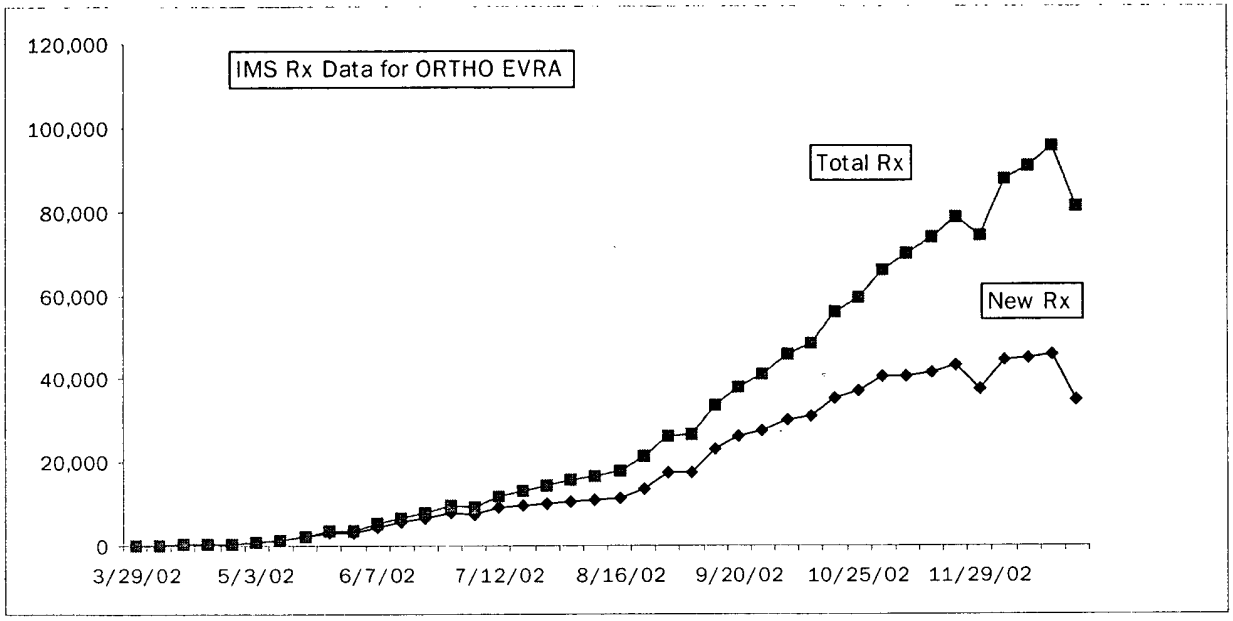
IMS RX DATA FOR ORTHO CYCLEN – NEW PRESCRIPTIONS



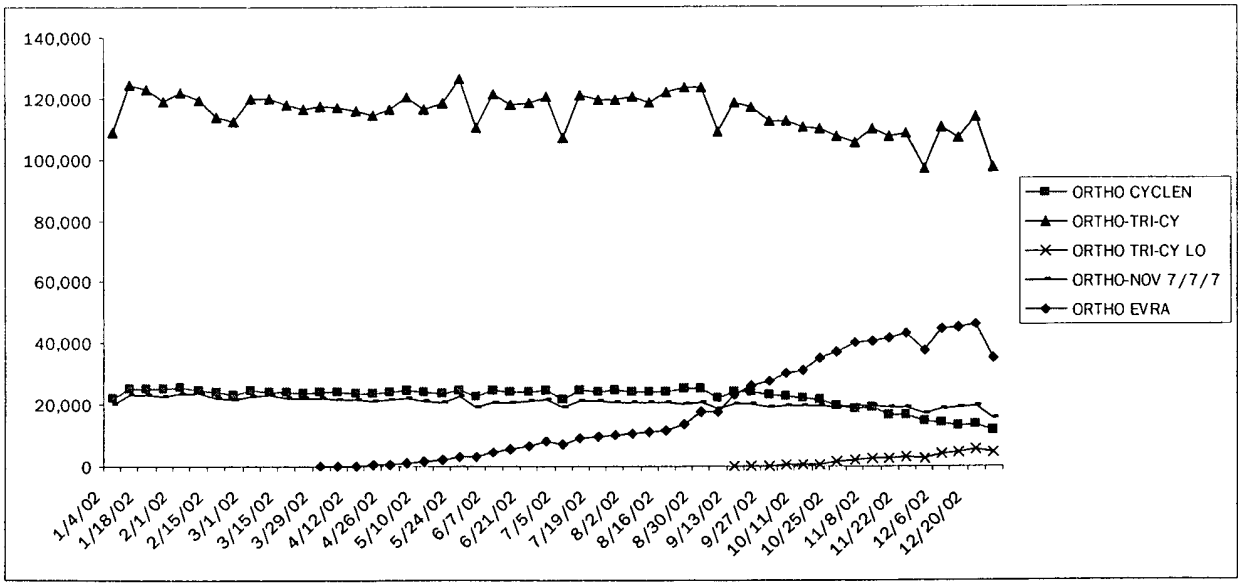
IMS RX DATA FOR ORTHO CYCLEN – TOTAL PRESCRIPTIONS



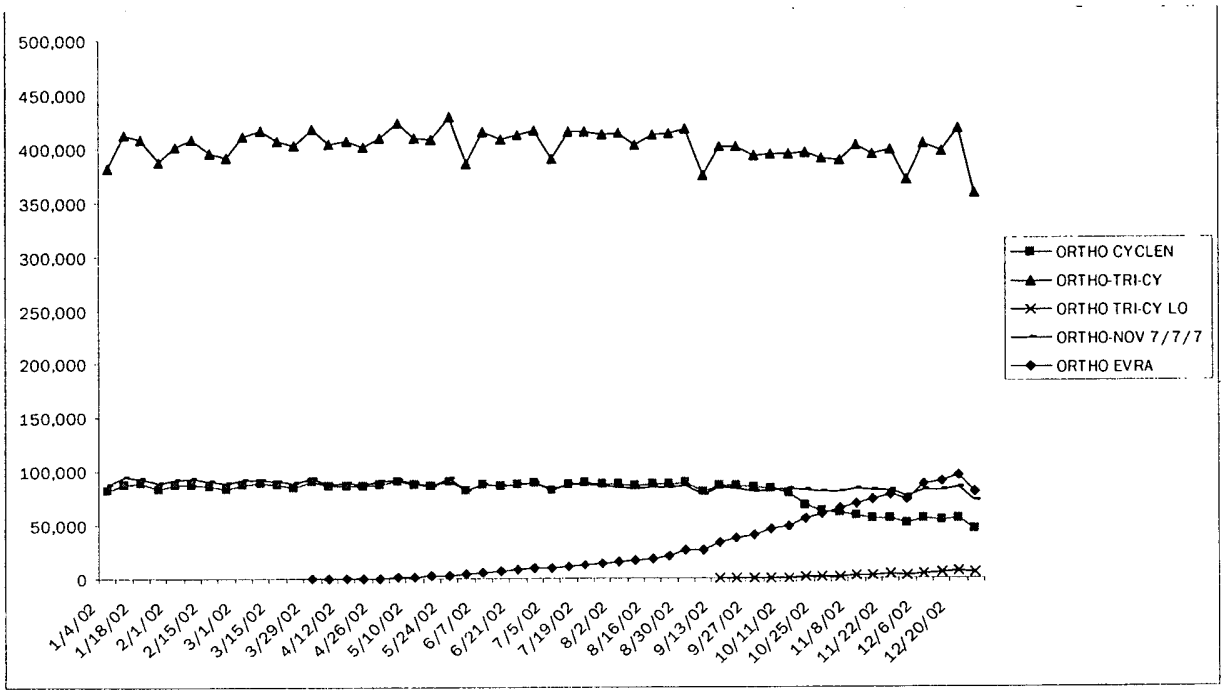
On a positive note, prescription growth for Ortho Evra has accelerated significantly since August – with total prescriptions growing from 15,000 to close to 100,000 exiting 2002, and new prescription growth remaining robust. The current Rx run rate exiting 2002 is over $120MM in sales which is ahead of our expectations and puts Evra in a solid number 2 spot with the Ortho Contraceptives franchise heading into 2003.



IMS RX DATA – NEW RX OF KEY ORTHO CONTRACEPTIVE FRANCHISES



IMS RX DATA – TOTAL RX OF KEY ORTHO CONTRACEPTIVE FRANCHISES



	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	Q2 A	Q3 A	Q4 A	Year	Q1A	Q2 A	Q3 A	Q4 E	Year	Q1E	Q2 E	Q3 E	Q4 E	Year
Worldwide															
Med. Device & Diag.	2,735	2,785	2,772	2,854	11,146	2,958	3,166	3,141	3,158	12,423	3,249	3,702	3,729	3,758	14,438
Pharmaceutical	3,490	3,864	3,677	3,820	14,851	4,180	4,258	4,277	4,443	17,159	4,568	4,634	4,791	4,966	18,959
Base J&J	3,260	3,609	3,398	3,563	13,830	3,895	3,974	3,931	4,107	15,907	4,218	4,296	4,368	4,547	17,429
Alza	230	255	280	257	1,022	286	284	346	336	1,252	350	338	424	419	1.531
Consumer	1,630	1,530	1,609	1,551	6,320	1,604	1,649	1,661	1,599	6,513	1,651	1,694	1,704	1,643	6,692
WW Total	**7,855**	**8,179**	**8,058**	**8,225**	**32,317**	**8,743**	**9,073**	**9,079**	**9,200**	**36,095**	**9,468**	**10,030**	**10,225**	**10,367**	**40,090**
U.S.															
Med. Device & Diag.	1,463	1,530	1,569	1,574	6,136	1,663	1,758	1,740	1,738	6,899	1,846	2,174	2,226	2,236	8,482
Pharmaceutical	2,356	2,722	2,511	2,651	10,240	2,958	2,934	2,939	3,155	11,986	3,225	3,172	3,308	3,543	13,248
Base J&J	2,126	2,467	2,232	2,394	9,219	2,672	2,650	2,593	2,818	10,733	2,875	2,834	2,884	3,123	11,717
Alza	230	255	280	257	1,022	286	284	346	336	1,252	350	338	424	419	1,531
Consumer	896	807	896	850	3,449	900	907	910	883	3,600	929	935	936	906	3,706
U.S. Total	**4,715**	**5,059**	**4,976**	**5,075**	**19,825**	**5,521**	**5,599**	**5,589**	**5,776**	**22,485**	**6,000**	**6,281**	**6,470**	**6,685**	**25,437**
International															
Med. Device & Diag.	1,272	1,255	1,203	1,280	5,010	1,295	1,408	1,401	1,419	5,523	1,403	1,527	1,503	1,522	5,956
Pharmaceutical	1,134	1,142	1,166	1,169	4,611	1,223	1,324	1,338	1,289	5,173	1,343	1,462	1,484	1,423	5,711
Consumer	735	723	713	701	2,872	704	742	751	716	2,913	722	760	768	736	2,986
Int. Total	**3,141**	**3,120**	**3,082**	**3,150**	**12,493**	**3,221**	**3,474**	**3,490**	**3,424**	**13,610**	**3,468**	**3,749**	**3,755**	**3,682**	**14,653**
Growth Analysis															
Worldwide															
Med. Device & Diag.						8.2%	13.7%	13.3%	10.6%	11.5%	9.8%	16.9%	18.7%	19.0%	16.2%
Pharmaceutical						19.8%	10.2%	16.3%	16.3%	15.5%	9.3%	8.8%	12.0%	11.8%	10.5%
Base J&J						19.5%	10.1%	15.7%	15.3%	15.0%	8.3%	8.1%	11.1%	10.7%	9.6%
Alza						24.2%	11.5%	23.8%	30.9%	22.6%	22.3%	18.9%	22.5%	24.7%	22.2%
Consumer						-1.6%	7.8%	3.2%	3.1%	3.1%	2.9%	2.7%	2.6%	2.7%	2.8%
WW Total						**11.3%**	**10.9%**	**12.7%**	**11.9%**	**11.7%**	**8.3%**	**10.5%**	**12.6%**	**12.7%**	**11.1%**
U.S.															
Med. Device & Diag.						13.7%	14.9%	10.9%	10.4%	12.4%	11.0%	23.7%	27.9%	28.6%	22.9%
Pharmaceutical						25.6%	7.8%	17.0%	19.0%	17.0%	9.0%	8.1%	12.6%	12.3%	10.5%
Base J&J						25.7%	7.4%	16.2%	17.7%	16.4%	7.6%	7.0%	11.2%	10.8%	9.2%
Alza						24.2%	11.5%	23.8%	30.9%	22.6%	22.3%	18.9%	22.5%	24.7%	22.2%
Consumer						0.5%	12.4%	1.6%	3.9%	4.4%	3.2%	3.1%	2.9%	2.7%	3.0%
U.S. Total						**17.1%**	**10.7%**	**12.3%**	**13.8%**	**13.4%**	**8.7%**	**12.2%**	**15.8%**	**15.7%**	**13.1%**
International															
Med. Device & Diag.						1.8%	12.2%	16.5%	10.9%	10.2%	8.3%	8.5%	7.3%	7.3%	7.8%
Pharmaceutical						7.8%	15.9%	14.8%	10.3%	12.2%	9.8%	10.4%	10.9%	10.4%	10.4%
Consumer						-4.2%	2.6%	5.3%	2.2%	1.4%	2.6%	2.4%	2.2%	2.8%	2.5%
Int. Total						**2.6%**	**11.3%**	**13.2%**	**8.7%**	**8.9%**	**7.6%**	**7.9%**	**7.6%**	**7.5%**	**7.7%**

Source: Company reports and SG Cowen estimates.

	Fiscal Year Ending December 2001					Fiscal Year Ending December 2002					Fiscal Year Ending December 2003				
	Q1A	**Q2 A**	**Q3 A**	**Q4 A**	**Year**	**Q1A**	**Q2 A**	**Q3 A**	**Q4 E**	**Year**	**Q1E**	**Q2 E**	**Q3 E**	**Q4 E**	**Year**
Total Revenue	**7,855**	**8,179**	**8,058**	**8,225**	**32,317**	**8,743**	**9,073**	**9,079**	**9,200**	**36,095**	**9,468**	**10,030**	**10,225**	**10,367**	**40,090**
Cost of Goods Sold	2,311	2,372	2,396	2,502	9,581	2,457	2,582	2,611	2,753	10,403	2,651	2,824	2,930	3,050	11,456
Gross Profit	**5,544**	**5,807**	**5,662**	**5,723**	**22,736**	**6,286**	**6,491**	**6,468**	**6,447**	**25,692**	**6,816**	**7,206**	**7,295**	**7,317**	**28,634**
R&D	759	829	899	1,104	3,591	831	932	952	1,272	3,987	947	1,045	1,099	1,458	4,549
SG&A	2,666	2,802	2,703	3,089	11,260	2,843	3,017	3,006	3,313	12,179	3,022	3,265	3,365	3,639	13,291
Operating Income	**2,119**	**2,176**	**2,060**	**1,530**	**7,885**	**2,612**	**2,542**	**2,510**	**1,863**	**9,527**	**2,847**	**2,895**	**2,831**	**2,220**	**10,793**
Interest	(92)	(70)	(67)	(74)	(303)	(42)	(30)	(12)	(20)	(104)	(28)	(40)	(55)	(65)	(188)
Other	(6)	15	(18)	55	46	33	(45)	129	(20)	97	(15)	(15)	(15)	(15)	(60)
Pretax Income	**2,217**	**2,231**	**2,145**	**1,549**	**8,142**	**2,621**	**2,617**	**2,393**	**1,903**	**9,534**	**2,890**	**2,950**	**2,901**	**2,300**	**11,041**
Taxes	665	647	579	339	2,230	787	774	668	476	2,705	824	841	827	655	3,147
Net Income	**1,552**	**1,584**	**1,566**	**1,210**	**5,912**	**1,834**	**1,843**	**1,725**	**1,427**	**6,829**	**2,066**	**2,110**	**2,074**	**1,644**	**7,894**
Net Interest Add Back*	9	9	0	0	18	0	0	0	0	0	0	0	0	0	0
Adj. Net Income	**1,561**	**1,593**	**1,566**	**1,210**	**5,930**	**1,834**	**1,843**	**1,725**	**1,427**	**6,829**	**2,066**	**2,110**	**2,074**	**1,644**	**7,894**
Shares (MM)	3,107	3,111	3,111	3,121	3,112	3,115	3,069	3,027	3,027	3,060	3,030	3,031	3,033	3,035	3,032
Earnings Per Share	**$0.50**	**$0.51**	**$0.50**	**$0.39**	**$1.91**	**$0.59**	**$0.60**	**$0.57**	**$0.47**	**$2.23**	**$0.68**	**$0.70**	**$0.68**	**$0.54**	**$2.60**
Margin Analysis															
Gross Profit	70.6%	71.0%	70.3%	69.6%	70.4%	71.9%	71.5%	71.2%	70.1%	71.2%	72.0%	71.8%	71.3%	70.6%	71.4%
R&D	9.7%	10.1%	11.2%	13.4%	11.1%	9.5%	10.3%	10.5%	13.8%	11.0%	10.0%	10.4%	10.7%	14.1%	11.3%
SG&A	33.9%	34.3%	33.5%	37.6%	34.8%	32.5%	33.3%	33.1%	36.0%	33.7%	31.9%	32.6%	32.9%	35.1%	33.2%
Operating Income	27.0%	26.6%	25.6%	18.6%	24.4%	29.9%	28.0%	27.6%	20.3%	26.4%	30.1%	28.9%	27.7%	21.4%	26.9%
Pretax Income	28.2%	27.3%	26.6%	18.8%	25.2%	30.0%	28.8%	26.4%	20.7%	26.4%	30.5%	29.4%	28.4%	22.2%	27.5%
Net Income	19.8%	19.4%	19.4%	14.7%	18.3%	21.0%	20.3%	19.0%	15.5%	18.9%	21.8%	21.0%	20.3%	15.9%	19.7%
Tax Rate	30.0%	29.0%	27.0%	21.9%	27.4%	30.0%	29.6%	27.9%	25.0%	28.4%	28.5%	28.5%	28.5%	28.5%	28.5%
Growth Analysis															
Total Revenue						11.3%	10.9%	12.7%	11.9%	11.7%	8.3%	10.5%	12.6%	12.7%	11.1%
Gross Profit						13.4%	11.8%	14.2%	12.7%	13.0%	8.4%	11.0%	12.8%	13.5%	11.5%
R&D	12.1%	16.3%	21.7%	13.1%	15.7%	9.5%	12.4%	5.9%	15.2%	11.0%	14.0%	12.2%	15.4%	14.6%	14.1%
SG&A						6.6%	7.7%	11.2%	7.2%	8.2%	6.3%	8.2%	11.9%	9.9%	9.1%
Operating Income	15.0%	16.6%	18.3%	31.3%	19.2%	23.2%	16.8%	21.8%	21.8%	20.8%	9.0%	13.9%	12.8%	19.1%	13.3%
Pretax Income	15.8%	16.6%	17.0%	24.9%	18.0%	18.2%	17.3%	11.6%	22.9%	17.1%	10.3%	12.7%	21.2%	20.8%	15.8%
Net Income	16.6%	16.2%	18.3%	23.3%	18.3%	18.1%	16.4%	10.2%	18.0%	15.5%	12.7%	14.5%	20.2%	15.2%	15.6%
EPS	14.2%	15.4%	17.6%	22.0%	17.0%	17.1%	17.3%	13.2%	21.6%	17.1%	15.9%	15.9%	20.0%	14.9%	16.6%

Source: Company reports and SG Cowen estimates.

Estimated Annual Revenue ($MM)

	FY2000	2001	2002E	2003E	2004E	2005E	2006E	01-06E Annual Growth Rate	% 00 - 01	% 01 - 02	% 02 - 03	% 03 - 04	% 04 - 05	% 05 - 06
Total Divisional Summary	$29,847	$32,417	$36,095	$40,090	$43,306	$47,111	$50,817	9%	9%	11%	11%	8%	9%	8%
Domestic	17,708	19,869	22,486	25,437	27,455	30,083	32,578	10%	12%	13%	13%	8%	10%	8%
International	12,139	12,548	13,609	14,653	15,851	17,028	18,239	8%	3%	8%	8%	8%	7%	7%
Med Device & Diag.	**10,281**	**11,211**	**12,423**	**14,438**	**15,500**	**16,907**	**18,103**	10%	9%	11%	16%	7%	9%	7%
Domestic	5,506	6,181	6,900	8,482	9,028	9,944	10,671	12%	12%	12%	23%	6%	10%	7%
International	4,775	5,030	5,523	5,956	6,472	6,964	7,432	8%	5%	10%	8%	9%	8%	7%
Pharmaceutical	**12,662**	**14,851**	**17,159**	**18,960**	**20,866**	**22,998**	**25,249**	11%	17%	16%	10%	10%	10%	10%
Domestic	8,442	10,240	11,986	13,248	14,561	16,102	17,708	12%	21%	17%	11%	10%	11%	10%
International	4,220	4,611	5,173	5,711	6,306	6,896	7,542	10%	9%	12%	10%	10%	9%	9%
Consumer	**6,904**	**6,356**	**6,513**	**6,692**	**6,939**	**7,206**	**7,464**	3%	-8%	2%	3%	4%	4%	4%
Domestic	3,760	3,449	3,600	3,706	3,866	4,037	4,199	4%	-8%	4%	3%	4%	4%	4%
International	3,144	2,907	2,913	2,986	3,073	3,168	3,265	2%	-8%	0%	3%	3%	3%	3%

Johnson & Johnson Estimated Annual Profit and Loss Statement ($MM)

	FY2000	2001	2002E	2003E	2004E	2005E	2006E	01-06E Annual Growth Rate
Total Revenues	**29,846**	**32,317**	**36,095**	**40,090**	**43,306**	**47,111**	**50,817**	**9.5%**
Cost of Goods Sold	8,908	9,581	10,403	11,456	12,202	13,132	13,962	
Gross Profit	**20,938**	**22,736**	**25,692**	**28,634**	**31,104**	**33,979**	**36,855**	**10.1%**
R&D	3,105	3,591	3,987	4,549	5,022	5,582	5,970	
SG&A	11,218	11,260	12,179	13,291	14,003	14,818	15,527	
Operating Income	**6,615**	**7,885**	**9,527**	**10,793**	**12,079**	**13,579**	**15,358**	**14.3%**
Interest, Net	(225)	(303)	(104)	(188)	(385)	(555)	(630)	
Other, Net	(61)	46	97	(60)	(60)	(60)	(60)	
Pretax Income	**6,901**	**8,142**	**9,534**	**11,041**	**12,524**	**14,194**	**16,048**	**14.5%**
Taxes	1,903	2,230	2,705	3,147	3,569	4,045	4,574	
Net Income	**4,998**	**5,912**	**6,829**	**7,894**	**8,955**	**10,148**	**11,474**	**14.2%**
Net Int. Add Back	**45**	**18**	0	0	0	0	0	
Adj. Net Income	**5,043**	**5,930**	**6,829**	**7,894**	**8,955**	**10,148**	**11,474**	**14.1%**
Shares (MM)	3,097	3,112	3,060	3,032	3,032	3,032	3,032	
Earnings Per Share	**$1.63**	**$1.91**	**$2.23**	**$2.60**	**$2.95**	**$3.35**	**$3.78**	**14.7%**
Growth Analysis								
Total Revenues	6.6%	8.3%	11.7%	11.1%	8.0%	8.8%	7.9%	
Gross Profit	7.3%	8.6%	13.0%	11.5%	8.6%	9.2%	8.5%	
R&D	12.2%	15.7%	11.0%	14.1%	10.4%	11.1%	7.0%	
SG&A	4.3%	0.4%	8.2%	9.1%	5.4%	5.8%	4.8%	
Operating Income	10.5%	19.2%	20.8%	13.3%	11.9%	12.4%	13.1%	
Pretax Income	17.0%	18.0%	17.1%	15.8%	13.4%	13.3%	13.1%	
Net Income	17.7%	18.3%	15.5%	15.6%	13.4%	13.3%	13.1%	
Earnings Per Share	18.9%	17.0%	17.1%	16.6%	13.4%	13.3%	13.1%	
Margin Analysis								
Gross Margin	70.2%	70.4%	71.2%	71.4%	71.8%	72.1%	72.5%	
R&D	10.4%	11.1%	11.0%	11.3%	11.6%	11.8%	11.7%	
SG&A	37.6%	34.8%	33.7%	33.2%	32.3%	31.5%	30.6%	
Operating Income	22.2%	24.4%	26.4%	26.9%	27.9%	28.8%	30.2%	
Pretax Income	23.1%	25.2%	26.4%	27.5%	28.9%	30.1%	31.6%	
Net Income	16.7%	18.3%	18.9%	19.7%	20.7%	21.5%	22.6%	
Tax Rate	27.6%	27.4%	28.4%	28.5%	28.5%	28.5%	28.5%	

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SG COWEN RATING DEFINITIONS

Rating	Definition
Strong Buy (1)	Stock expected to outperform the market by over 25%
Outperform (2)	Stock expected to outperform the market by 10-25%
Market Perform (3)	Stock expected to out/underperform the market by +/-10%
Underperform (4)	Stock expected to underperform the market by at least 10%

Assumptions: Time horizon is 12 months; market is flat over forecast period.

SG COWEN RATING DEFINITIONS PRIOR TO 9/9/2002

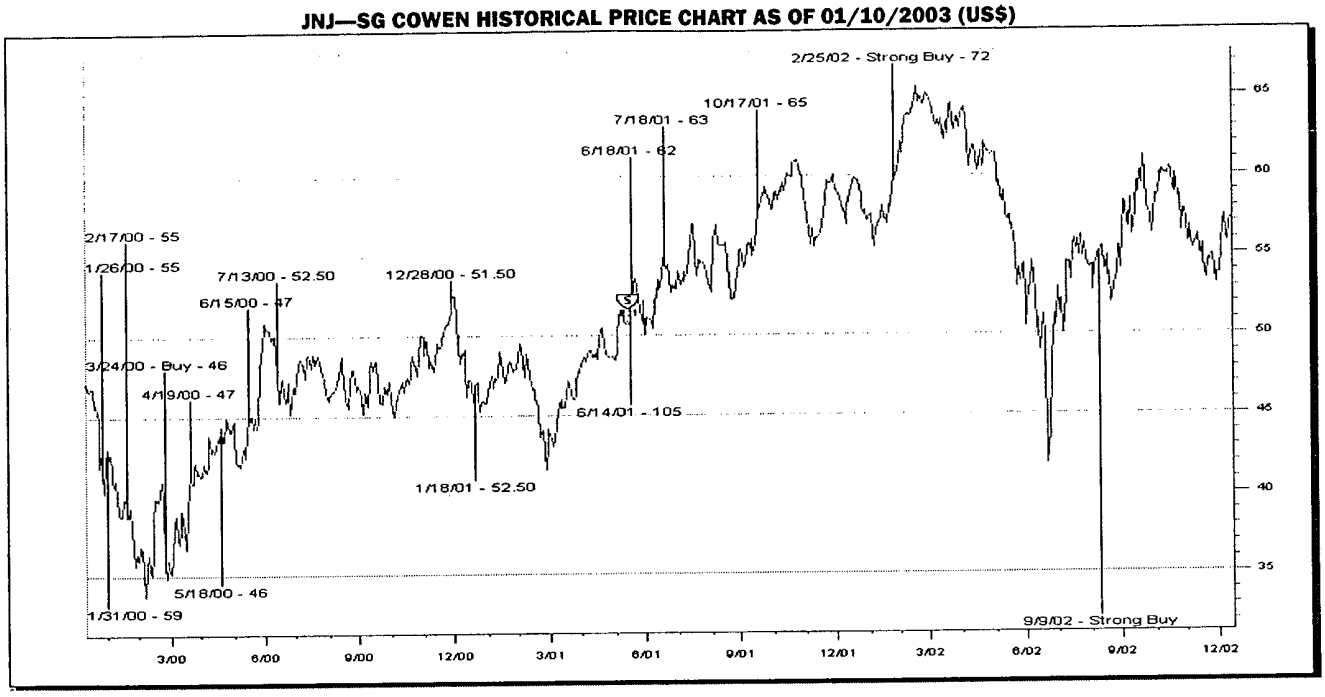
Rating	Definition
Strong Buy (1)	Analyst expects the stock to outperform the market over the next 6-12 months
Buy (2)	Analyst expects the stock to outperform the market over the next 12-18 months
Neutral (3)	Analyst expects the stock to perform in line with the market over the next 12 months
Underperform (4)	Analyst expects the stock to underperform the market over the next 12 months

SG COWEN RATING ALLOCATION (a)

Rating	Pct of companies under coverage with this rating	Pct for which Investment Banking services have been provided within the past 12 months
Buy (b)	54.1%	6.6%
Hold (c)	39.5%	1.4%
Sell (d)	6.4%	0.6%

(a) As of 12/31/2002. (b) Includes "Strong Buy" and "Outperform" rated stocks as defined in the SG Cowen's rating system (see above). (c) Corresponds to "Market Perform" as defined in the SG Cowen's ratings system (see above). (d) Corresponds to "Underperform" as defined in the SG Cowen's ratings system (see above).
Note: "Buy," "Hold" and "Sell" are not defined SG Cowen ratings and should not be construed as investment options. Rather, these ratings are used illustratively to comply with NASD and NYSE regulations.

JNJ—SG COWEN HISTORICAL PRICE CHART AS OF 01/10/2003 (US$)



Initiated on 05/01/94 with Neutral ; Rating as of 12/04/98 was Strong Buy; Price Target as of 08/05/99 was $59.00

February 13, 2003

Amgen Inc (AMGN - $52.32) 1-Overweight

Company Update

NEJM; Is Remicade efficacious long term?

United States
Healthcare
Biotechnology
Craig C. Parker
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Investment conclusion

- The NEJM has published data questioning the long term efficacy of Remicade when it is used without concomitant immunosuppressive therapy. JNJ's decision to use Remicade® monotherapy for psoriasis and psoriatic arthritis may have been short sighted. We believe these data reinforce our position that Amgen's Enbrel will be the dominant TNF antagonist. We reiterate our Overweight rating.

Summary

- Antibodies against Remicade are associated with infusion reactions and a reduced duration of efficacy.
- Concurrent immunosuppression reduces the formation of antibodies, reduces the risk of infusion reactions and increases duration of efficacy.
- We question the rationale of pursuing new indications, especially psoriasis, without concurrent immunosuppressive therapy.

EPS (FY Dec)

	2002	2003			2004			% Change	
	Actual	Old	New	St. Est	Old	New	St. Est	2003	2004
1Q	0.32	0.41E	0.41E	0.39E	NA	NA	0.49E	28	NA
2Q	0.38	0.43E	0.43E	0.43E	NA	NA	0.51E	13	NA
3Q	0.34	0.46E	0.46E	0.45E	NA	NA	0.51E	35	NA
4Q	0.35	0.49E	0.49E	0.48E	NA	NA	0.53E	40	NA
Year	1.39	1.78E	1.78E	1.75E	NA	2.20E	2.13E	28	24
P/E			29.4			23.8			

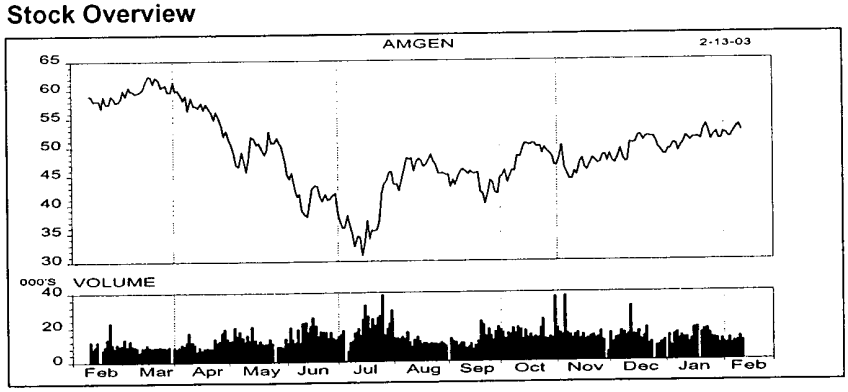
Market Data

Market Cap	68157.3M
Shares Outstanding (Mil)	1302.7
Float	NA
Dividend Yield	NA
Convertible	Yes
52 wk Range	62.94 - 30.57

Financial Summary

Revenue FY03	$7.5B
Five-Year EPS CAGR	22.00
Return on Equity	NA
Current BVPS	1.5
Debt To Capital	99.8

Stock Overview



Stock Rating:	Target:
New: 1-Overweight	New: 61.00
Old: 1-Overweight	Old: 61.00

Sector View: 1-Positive

Introduction

The February 13 issue of the NEJM publishes data on Remicade antigenicity as an "Original Article". The paper entitled "Influence of Immunogenicity on the Long-Term Efficacy of Infliximab in Crohn's Disease" is we believe a harbinger of what we predict will happen in both psoriasis and psoriatic arthritis. Crohn's Disease was the first approved indication for Remicade and the product is without question highly efficacious even as a single infusion. Not surprisingly efficacy from a single infusion, or short 3-infusion course, of Remicade wanes but the disease has been shown to respond to additional infusions. Remicade is a chimeric anti-TNF antibody and as such contains non-human protein sequences which can be recognized as foreign. The immune system treats this foreign protein like any other and antibodies form that recognize and bind to this protein, targeting it for destruction. These antibodies can be either neutralizing, i.e. they bind to the active site, which in this case would inhibit or reduce the capacity of Remicade to bind to TNF, or non-neutralizing in which case they attach to the scaffolding part of the molecule. In either case there is the potential for both pharmacokinetics and pharmacodynamics to change, which may impact efficacy; this can clearly be exacerbated when the therapeutic has a long half life and is dosed intermittently as is the case with Remicade. The use of concurrent immunosuppressive agents, such as methotrexate, reduce the formation of these anti-Remicade antibodies.

In clinical practice severe Crohn's Disease patients may have run the gamut of alternative therapeutics and Remicade is often used as a single agent. In the treatment of RA the use of methotrexate as a gold standard led to the clinical development of Remicade in combination with methotrexate. Based on what has been described above therefore we would expect to see a greater problem with anti-Remicade antibodies in Crohn's Disease patients than RA patients. We would also predict that new indications for Remicade would be similarly affected depending on the presence or absence of concurrent immunosuppressive therapy. Data emerging in 2002 showed that Remicade is very effective in the treatment of both psoriasis and psoriatic arthritis, in fact the skin responses observed with Remicade so

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far suggest that it has the most potent efficacy of all the biologics in development. These studies used Remicade monotherapy and we believe that JNJ is moving toward pivotal studies for both indications with monotherapy. Thus we believe that there is a strong likelihood that long-term use antigenicity data for Remicade in both psoriasis and psoriatic arthritis will mirror those discussed in the current NEJM article, particularly for psoriasis where efficacy data to date suggest intermittent therapy is feasible. The use of Remicade in combination with immunosuppressive therapy would reduce its attractiveness to both dermatologists and patients.

At this juncture it is important to point out that Amgen's Enbrel, Biogen's Amevive®, Genentech's Raptiva™ and Abbott's Humira® are all either fully human or humanized, in the case of Raptiva and thus we would not anticipate antigenicity of these molecules to be significant.

Method Summary

The paper describes results from 125 moderate-severe Crohn's Disease patients treated at one of 2 centers in Belgium; all assays were performed by Prometheus Laboratories using validated commercial assays. Forty five percent of these patients were taking concurrent immunosuppressive therapy and 55% were not; the median duration of follow-up in the study was 36 months. Patients received the standard regimen of 5 mg/kg dosed at weeks 0, 2 and 6 with a second course initiated upon relapse. Sera were taken prior to each infusion, 4 weeks after each infusion and at any visit and were assayed for Remicade concentration and the presence of antibodies to Remicade. In addition early and late infusions-related reactions were recorded.

Results Summary

At baseline no patients were positive for antibodies to Remicade, however, after the 5th infusion 61% were antibody positive. At an antibody concentration of > 8 µg/mL, seen in 37% of patients, more infusion reactions and a shorter duration of response was observed. The table below summarizes the impact of immunosuppression.

Immunosuppression	N	% +ve for anti-Remicade antibodies	Antibody Concentrations	
			Fistulizing	Non-fistulizing
Present	54	43%	1.5	1.3
None	71	75%	21.4	13.8

The presence of immunosuppression was statistically significant not only for the presence or absence of anti-Remicade antibodies but for the antibody titer. The latter is important as the median antibody concentration was 20.1 µg/mL for patients having an infusion reaction versus 3.2 µg/mL for those not having a reaction. Further antibody titers predicted the duration of response; 71 days of those patients with an antibody titer < 8 µg/mL versus 35 days at a concentration of > 8 µg/mL, again statistically significant.

Not surprisingly there was a positive correlation between anti-Remicade antibody titer and Remicade concentration in serum. The overall median Remicade concentration 4 weeks following an infusion was 12.0 µg/mL. Patients taking immunosuppressive therapies were more likely to have a Remicade serum concentration > 12 µg/mL than those not taking immunosuppressive therapies, again highly statistically significant. Further Remicade concentration 4 weeks following infusion was correlated to the presence or absence of an infusion reaction which in turn predicted the duration of response.

- The median duration of response was 38.5 days in those patients experiencing an infusion reaction compared to 65 days in those not experiencing such a reaction. Whilst further infusion reactions could be ameliorated or prevented with premedication the duration of response remained low.
- Patients with a Remicade serum concentration of >12 µg/mL had a median duration of response of 81.5 days versus 68.5 days for patients with Remicade concentrations <12 µg/mL.

Valuation: Our $61 12-month price target is based on the application of a 34 x PE multiple to our 2003 EPS estimate of $1.78.

Company Description:

Amgen is a leading global biotechnology company that dominates the market for blood cell stimulating drugs. Targets for future products include oncology, infectious disease and neurology.

Company Name:	Disclosures	Ticker	Price (02/12)	Rating
Amgen Inc	C	AMGN	52.32	1-Overweight
Related Tickers:	**Disclosures**	**Ticker**	**Price (02/12)**	**Rating**
Biogen Inc	C,D,E	BGEN	37.76	1-Overweight
Genentech Inc	E	DNA	35.35	1-Overweight
Johnson & Johnson		JNJ	50.00	0-Not Rated

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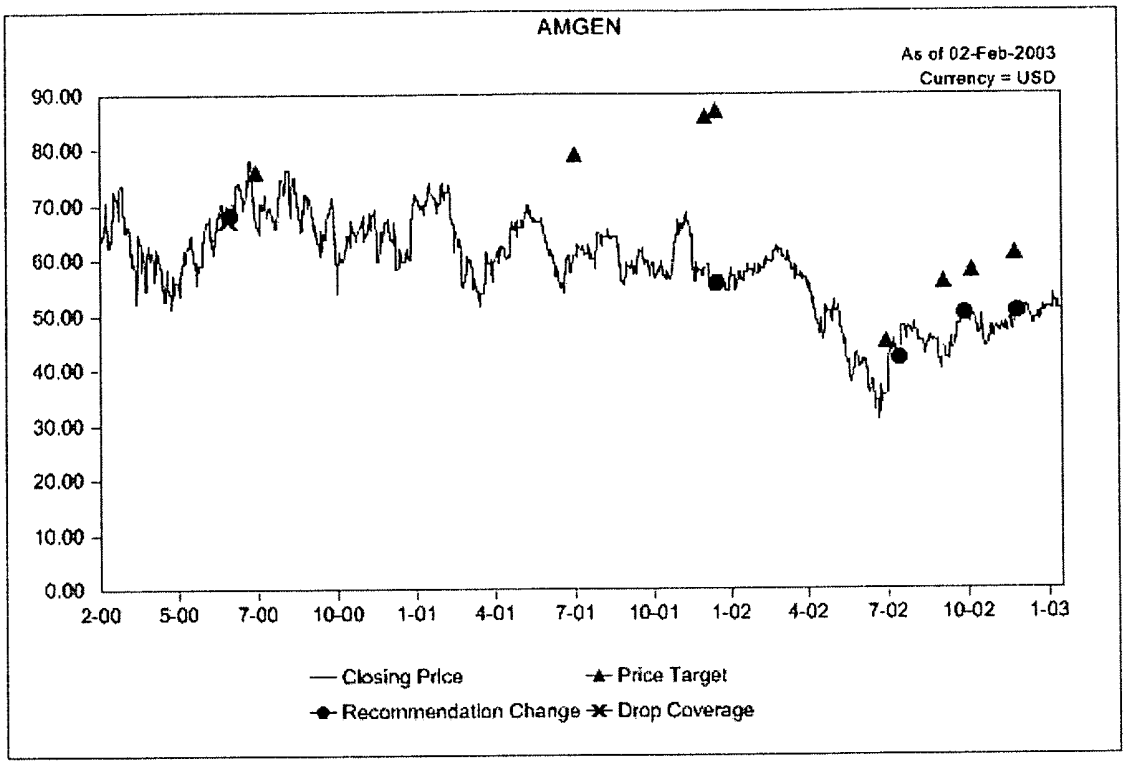
Important Disclosures

Rating and Price Target Chart: AMGN



Date	Closing Price	Rating	Price Target
12-Dec-02	$50.45	1-Overweight	
12-Dec-02	$50.45		$61.00
24-Oct-02	$48.68		$58.00
17-Oct-02	$50.15	1-Overweight	
24-Sep-02	$42.07		$56.00
05-Aug-02	$42.22	1-Overweight	
22-Jul-02	$35.28		$45.00

Date	Closing Price	Rating	Price Target
07-Jan-02	$55.69	1-Strong Buy	
07-Jan-02	$55.69		$87.00
27-Dec-01	$58.40		$86.00
29-Jul-01	$60.82		$79.00
28-Jul-00	$66.38		$76.00
29-Jun-00	$68.25	2-Outperform	
27-Jun-00	$67.38	Dropped	

FOR EXPLANATION OF RATINGS PLEASE REFER TO THE STOCK RATING KEYS LOCATED AT THE END OF THIS DOCUMENT

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D - Lehman Brothers Inc. and/or an affiliate has received compensation for investment banking services from the subject company within the past 12 months.
E - Lehman Brothers Inc. and/or an affiliate expects to receive or intends to seek compensation for investment banking services from the subject company within the next 3 months.

Risk Disclosure:
AMGN: Changes in reimbursement and market penetration of currently marketed products could adversely affect the stock and impact our rating and price target. The Rhode Island manufacturing facility for Enbrel is assumed to gain approval by YE02 - early 2003, if there is a delay, our revenue assumptions may change due to manufacturing constraints and potential increased competition from Abbott's D2E7 should Abbott's drug gain approval early in 2003.

Key to Investment Opinions:

Stock Rating
1-Overweight - The stock is expected to outperform the unweighted expected total return of the industry sector over a 12-month investment horizon.
2-Equal weight - The stock is expected to perform in line with the unweighted expected total return of the industry sector over a 12-month investment horizon.
3-Underweight - The stock is expected to underperform the unweighted expected total return of the industry sector over a 12-month investment horizon.
RS-Rating Suspended - The rating and target price have been suspended temporarily to comply with applicable regulations and/or firm policies in certain circumstances including when Lehman Brothers is acting in an advisory capacity on a merger or strategic transaction involving the company.

Sector View
1-Positive - sector fundamentals/valuations are improving.
2-Neutral - sector fundamentals/valuations are steady, neither improving nor deteriorating.
3-Negative - sector fundamentals/valuations are deteriorating.

Stock Ratings From February 2001 to August 5, 2002 (sector view did not exist):
This is a guide to expected total return (price performance plus dividend) relative to the total return of the stock's local market over the next 12 months.
1-Strong Buy - expected to outperform the market by 15 or more percentage points.
2-Buy - expected to outperform the market by 5-15 percentage points.
3-Market Perform - expected to perform in line with the market, plus or minus 5 percentage points.
4-Market Underperform - expected to underperform the market by 5-15 percentage points.
5-Sell - expected to underperform the market by 15 or more percentage points.

Stock Ratings Prior to February 2001 (sector view did not exist):
1-Buy - expected to outperform the market by 15 or more percentage points.
2-Outperform - expected to outperform the market by 5-15 percentage points.
3-Neutral - expected to perform in line with the market, plus or minus 5 percentage points.
4-Underperform - expected to underperform the market by 5-15 percentage points.
5-Sell - expected to underperform the market by 15 or more percentage points.
V-Venture – return over multiyear timeframe consistent with venture capital; should only be held in a well diversified portfolio.

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Goldman Sachs

Johnson & Johnson (JNJ)

JNJ (IL/N) – Antibodies to Remicade are manageable according to NEJM study

Analyst Comment

12:05 PM – February 13, 2003

Stock rating: In-Line

Coverage view: Neutral

Large-Cap Growth

Price: US$50

United States

Stock data	
52-week range	US$65.49-41.85
Yield	1.7%

Price performance	1M	3M	12M
Absolute	-13%	-17%	-13%
Rel to S&P 500	-1%	-9%	13%

Capitalization	
Market cap	US$151.3bn
Latest net debt/(cash)	-
Free float	98%
Shares outstanding	3027mn

Forecasts/valuation	2003E	2004E
EPS*	US$2.63	US$2.95
GSCOPE EPS*	-	-
GSCOPE P/E	-	-

* May differ from US GAAP

We continue to rate Johnson & Johnson shares In-Line. Our coverage view of the medical device sector remains neutral. In Thursday's New England Journal of Medicine, a study examined the potential for formation of antibodies to J&J's Remicade (infliximab) in Crohn's disease pts. The study concluded that development of antibodies against Remicade is associated with an increased risk of infusion reactions and a reduced duration of response to treatment. However, the investigators clearly note that concomitant immunosuppressive therapy, such as methotrexate, prevents the formation of antibodies against Remicade. Consequently, immunosuppressive therapy initiated prior to Remicade treatment prevents infusion reactions and helps to maintain clinical efficacy. With Crohn's patients having limited options for treatment and the ability to prevent antibody formation with immunosuppressive treatment, we see little risk to changes in treatment with Remicade. As such, we believe that Remicade will continue to be a common treatment for refractory Crohn's disease.

We are maintaining our 2003 and 2004 EPS estimates of $2.63 and $2.95, respectively.

STUDY DESIGN. In this week's New England Journal of Medicine, investigators studied the formation of antibodies to Remicade in Crohn's disease patients. The study was conducted 125 consecutive patients with Crohn's disease, which were undergoing Remicade infusion therapy. Remicade concentration, antibodies to Remicade, clinical data, infusion reactions, and the use of concomitant immunosuppressive treatments were measured before and 4,8, and 12 weeks after each infusion.

RESULTS. In the series of patients, a mean of 3.9 infusions per patient were administered over a mean of 10 months. Antibodies to Remicade were detected in 61% of patients. The presence of antibody concentrations of 8.0ug per ml or greater before an infusion predicted a statistically significant shorter duration of response (35 days, as compared to 71 days among patients with concentrations of less than 8.0ug per ml) and a higher risk of infusion reactions. Concomitant immunosuppressive therapy was predictive of low titers of antibodies against Remicade ($p<0.001$) and high concentrations of Remicade 4-weeks after infusion ($p<0.001$).

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Goldman Sachs Research global coverage universe
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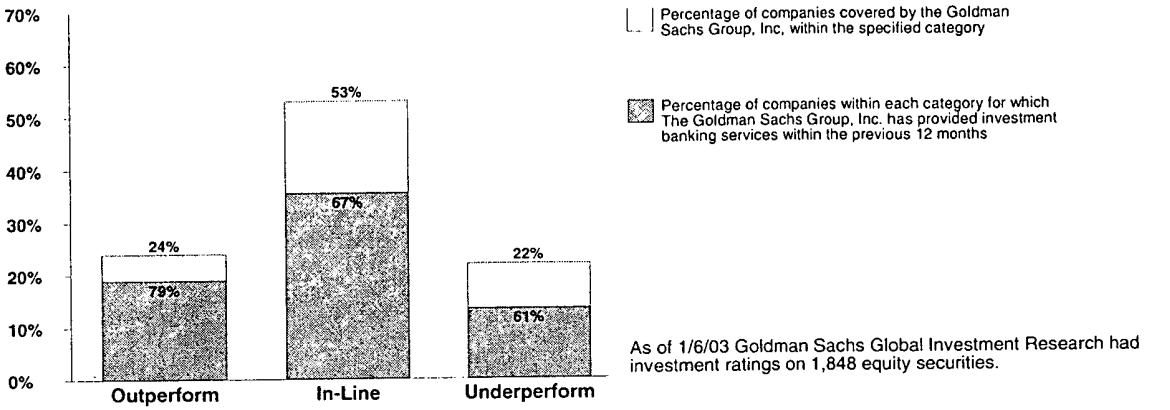


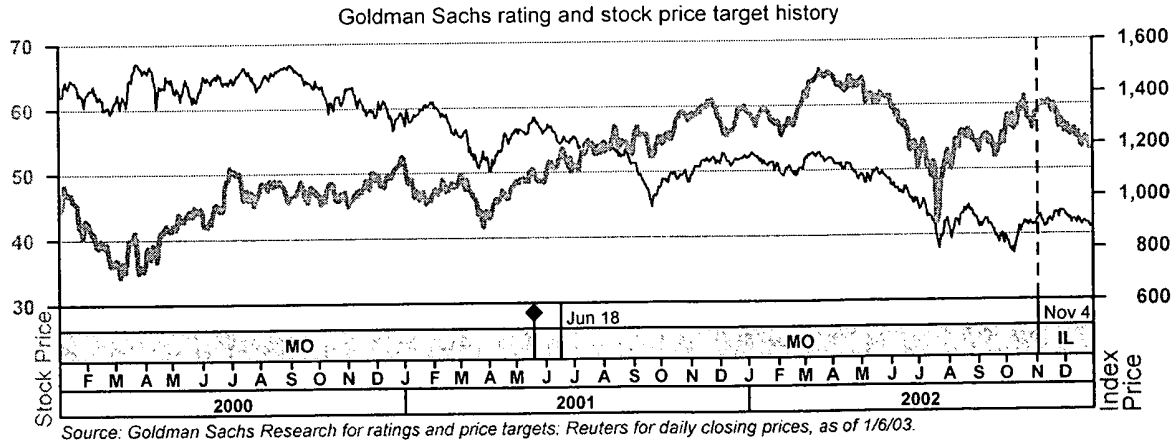
As of 1/6/03 Goldman Sachs Global Investment Research had investment ratings on 1,848 equity securities.

Goldman Sachs uses three ratings - Outperform, In-Line, and Underperform - reflecting expected stock price performance relative to each analyst's coverage group, on an unweighted basis with regard to market capitalization and with a 12-month time horizon. Each analyst also assigns a coverage view - Attractive, Neutral, or Cautious - representing the analyst's fundamental outlook on the coverage group. NASD/NYSE rules require a member to disclose the percentage of its rated securities to which the member would assign a buy, hold, or sell rating if such a system were used. Although relative ratings do not correlate to buy, hold, and sell ratings across all rated securities, for purposes of the NASD/NYSE rules, Goldman Sachs has determined the indicated percentages by assigning buy ratings to securities rated Outperform, hold ratings to securities rated In-Line, and sell ratings to securities rated Underperform, without regard to the coverage views of analysts.

Source: Goldman Sachs *As of January 6, 2003*

Johnson & Johnson (JNJ) Currency: U.S. Dollar

Goldman Sachs rating and stock price target history



Source: Goldman Sachs Research for ratings and price targets: Reuters for daily closing prices, as of 1/6/03.

◆ May 18, 2001 to NR from MO
☐ Rating
■ Price target
✕ Price target removal
— S&P 500; pricing by FactSet
Covered by Lawrence Keusch
Not covered by current analyst
New rating system as of 11/4/02

The price targets shown should be considered in the context of all prior published Goldman Sachs research, which may or may not have included price targets, as well as developments relating to the company, its industry and financial markets.

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Current rating system (as of November 4, 2002)

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Previous rating system

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LL = Latin America Recommended List. Expected to provide price gains at least 10 percentage points greater than the Latin America MSCI Index over the next 6-18 months.

TB = Trading Buy. Expected to provide price gains of at least 20 percentage points sometime in the next 6-9 months.

MO = Market Outperformer. Expected to provide price gains of at least 5-10 percentage points greater than the market over the next 6-18 months.

MP = Market Performer. Expected to provide price gains similar to the market over the next 6-18 months.

MU = Market Underperformer. Expected to provide price gains of at least 5 percentage points less than the market over the next 6-18 months.

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JNJ: US$ 50.00

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